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BIOLOGICAL BULLETIN

STUDIES IN ARTIFICIAL PARTHENOGENESIS.

II. Physical Changes in the Egg of Arbacia.

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Contents.

I. Physical Organization of the Egg.

In spite of the many researches on the sea-urchin egg very little is known of its physical make-up. Year after year the egg is used in attempts to analyze fundamental problems and various theories have been based upon experiments with it. And yet, but little is known definitely about the type of physical organization which it possesses. Is the egg essentially fluid or is it a more or less rigid jelly, is the unfertilized egg surrounded by a microscopically visible membrane or by a hypothetical film beyond the limits of vision, what indeed is the nature of the membrane which controls osmotic interchange? These

are some of the questions which must be considered if our theories are to be more than mere generalizations.

The first point to be decided upon is the physical nature of the egg contents. Embryologists in the past have often observed flowings in egg cytoplasm and such observations indicate a fluid composition. This would accord with Rhumbler's demonstration of fluidity in other types of protoplasm. We might accept these results without further comment if it were not for the fact that recently there has been a tendency to regard protoplasm as a gel. Kite ('13) has in fact concluded that the protoplasm of the starfish egg is of this nature. The protoplasm of the normal unfertilized sea-urchin egg is undoubtedly in a typically fluid condition. If pressure is applied to eggs underneath a coverslip, the egg contents flow out, indeed, if the pressure is vigorous enough, the protoplasm is shot out in a long stream as from a pipette. Facts such as these are probably known to many embryologists, similar observations have been especially described by Reinke ('95) and Albrecht ('98).

If the egg is essentially a fluid mass, what is there to prevent it from diffusing through the sea-water? Two possibilities exist, either (1) The substance of the egg is as a whole insoluble in sea-water or (2) it is surrounded by a membrane insoluble in sea-water.[1] The first possibility is excluded by the fact that we know protoplasm to be in aqueous solution. We must therefore conclude that the egg is surrounded by a membrane insoluble in sea-water. Careful observation reveals the existence of a membrane around the unfertilized egg; just outside of the darker substance of the egg cytoplasm, a dim outer line can usually be made out. The faintness with which the outer margin of this vitelline membrane appears is apparently due to the fact that its refractive index is very close to that of sea-water. That the appearance of a membrane is not the result of a diffraction illusion is shown by the fact that the membrane may be isolated by pressing out the egg contents, as Herbst first found. At fertilization, as will be pointed out more fully later, it is this

[1] The oft-made assumption of a surface film like that found at the surface of peptone solutions, etc., is really a special case of the first alternative. For such a film could only exist at a surface of discontinuity, and this could only occur at the junction of 2 immiscible fluids.

membrane which becomes lifted from the surface of the egg as the fertilization membrane.

A study of the chemical behavior of the membrane gave results of interest. Dilute acids cause it to swell. When one part of normal HCl is diluted with 9 parts of sea-water the resultant solution causes a marked swelling. The membrane becomes sticky and agglutination follows.[1] Dilute solutions of nitric, butyric, and valerianic acids give similar results. As far as can be ascertained however, this acid swelling of the membrane does not result in complete solution. On the other hand dilute alkaline solutions, although they cause little if any swelling of the membrane, are quite effective in dissolving it away. In order to study the effect of alkali on the membrane, it is best to shake the jelly off the eggs first, as this often becomes saturated with the Mg $(OH)_2$ precipitated by the alkali, and obscures the result. Eggs in 50 c.c. of sea-water plus 2.5 c.c. $n/10$ NaOH soon become sticky; they cling to the bottom of the dish and to each other. Soon the exterior surface of the egg becomes rough, it is evidently no longer surrounded by a membrane (and it is only prevented from diffusing through the sea-water by coagulation). Many salts exert a swelling effect on the membrane, and in some cases this is accompanied by a complete solution of the membrane. When eggs are dropped into 0.55 *N* NaI, the swollen membranes are very apparent after 10 or 15 minutes have elapsed. In 40 minutes almost all of the eggs are no longer surrounded by a membrane, the periphery of these eggs instead of being smooth, now presents a roughened appearance. It is quite evident that the protoplasm is naked and that the membrane has been completely dissolved away by the sodium iodide. This behavior of the membrane towards acids, alkalis, and salts, indicates its protein nature.

That it is not a lipoid is evident from the fact that it is insoluble in any of the ordinary lipoid solvents. It might however contain an admixture of lipoids. This is rendered improbable by the following line of evidence. As was pointed out above, the almost invisible character of the membrane indicates that its refractive

[1] Loeb ('08) described agglutination in HCl.

index is very close to that of sea-water. Now it is well known that many proteins have a refractive index close to that of sea-water; on the other hand, lipoids have a considerably higher refractive index. As the refractive index is an additive function of the constituents of a mixture, the presence of lipoids in any abundance would make it impossible for the refractive index of the membrane even to approach that of sea-water. Hence no great admixture of lipoids can be present. More direct evidence of the absence of any appreciable quantity of lipoids is also available. The membrane was tested with a Scharlach R solution such as recommended by Herxheimer. In order to render them more visible, the membranes were made to swell by placing the eggs in 15 c.c. sea-water plus 10 c.c. 2.5 *N* NaCl. To a drop of Scharlach R solution on a slide was added a drop of egg suspension. The vitelline membrane remained hyaline and unstained, whereas the egg cytoplasm itself was colored red. The Scharlach R solution used in this test was a saturated solution of the dye in equal parts of acetone and 70 per cent. alcohol.

If the egg contents be made to flow out from the membrane or if the egg be cut or shaken into fragments, a new membrane immediately forms around the momentarily naked protoplasm.[1] Such a membrane has the same chemical properties as the vitelline membrane. The acids and salts which produce swelling in the latter, cause it to swell also. The immediate production of a protein membrane about these egg fragments must be regarded as similar to the formation of precipitation membranes like those studied by Traube and Quincke. Evidently, some protein contained in the egg is precipitated on contact with the outer sea-water. It is probable that this protein is, in the interior of the egg, prevented from coagulation by the presence of a protecting colloid. At the outer surface of the egg, the membrane-protein is coagulated by direct contact with sea-water. Support for this view is found in the fact that the presence of some colloids (*e. g.*, egg albumen) in the sea-water, causes the membrane to take up water and swell. Since the vitelline mem-

[1] O. and R. Hertwig in their "Untersuchungen zur Morphologie und Physiologie der Zelle," Heft 5, 1887, first observed the elevation of membranes on egg fragments. (This observation was also repeated by Ziegler, Arch. f. Ent. Mech., VI., 249 (1898), by Moore, Univ. of California Publications in Physiology, IV., 89 (1912).

brane behaves toward reagents in the same manner as does the membrane on egg fragments, we are justified in regarding it too as a precipitation membrane. The most noteworthy feature of the precipitation membranes of Traube and Quincke is their semipermeable (or partially semi-permeable)[1] character. We should therefore expect the vitelline membrane to exhibit semipermeable properties, and in this way to govern osmotic interchange. When the egg is caused to shrink in a hypertonic solution, the vitelline membrane shrinks with it. This has been observed in a great variety of hypertonic solutions. For such a shrinkage, there are 3 possible types of explanation. The inward tension may be the result of a force arising from (1) the substances within the membrane, (2) the membrane itself, or (3) something immediately outside of the membrane.

Physico-chemically the possibilities, as I see them, are (1) The fluid interior of the egg is coagulated by the various hypertonic solutions, shrinks as a result and pulls the membrane with it. (2) The vitelline membrane as a result of its semipermeability[2] is responsible for the shrinkage. (3) There is an invisible semipermeable membrane outside of the vitelline membrane. As there is nothing to warrant the assumption of any semipermeable membrane outside of the vitelline membrane, it seems scarcely necessary to discuss the third possibility. The first possibility is essentially the position maintained by M. Fischer ('10). He regards the passage of water into and out of cells as due primarily to the attraction of the interior colloids for water. According to this view endosmosis, such as occurs in hypotonic solutions, would be the result of the taking up of water by colloids within the cell. That the process of water absorption is by no means dependent on the affinity of the egg colloids for water is conclusively proven by the fact that influx of water

[1] The existence of an absolutely semipermeable precipitation membrane (*i. e.*, one which prevents the passage of all substances in solution, but permits the passage of solvent) is extremely doubtful; *cf.* for example Quincke '02. The fact that a substance may penetrate a membrane and yet exert considerable osmotic pressure against it, has often been neglected by biologists. To assume that because a substance passes through a membrane it can exert no osmotic pressure against it, is just as foolish as to assume that the air in an air-bubble exerts no pressure on the film of water surrounding it.

[2] Using the term in its broadest sense.

leads not to transformation of gel to sol, as we would have to suppose, but on the contrary such an influx transforms the egg protoplasm from the sol to the gel condition. The experimental evidence in support of this fact is given on p. 195. *Water absorption by the egg is indeed correlated with a loss of water on the part of the egg proteins.* The passage of water into and out of the cell can not therefore be due to changes in the water content or aggregation state of the egg proteins, and the first possibility is unable to account for the facts. Only the second possibility remains, *i. e.*, that the vitelline membrane acts as a semipermeable membrane and controls osmotic intercourse. On the basis of this view many facts are understandable, which can be explained in no other way. This will, I think, be demonstrated in the course of the argument.

The plasma-membrane of a cell is defined as the membrane which governs its osmotic intercourse. According to this definition, *the vitelline membrane is the plasma-membrane of the Arbacia egg cell.* Hitherto no one has either described a plasma membrane, or studied directly the properties of one. Although he often uses the concept of such a membrane, Lepeschkin ('11) admits that the actual structure is "zurzeit unbekannt." Loewe, ('13) in referring to the plasma-membrane, says: "Allein weit davon entfernt, dasz auch nur ihre Existenz irgendwie sichergestellt wäre, sind auch über die Beschaffenheit dieses hypothetischen Gebildes die Meinungen so zahlreich wie die Möglichkeiten." Fischer ('12) is indeed of the opinion that "the entire conception of an osmotic membrane about cells is an impossibility."

The plasma-membrane of the *Arbacia* egg is a protein gel. As such, it possesses a certain degree of rigidity. Suppose a hypothetical system completely surrounded by an extremely rigid semipermeable membrane.[1] If such a system were placed in a concentrated solution no exosmosis could take place, for if the membrane were perfectly rigid, there could be no removal of solvent from the system without the production of a vacuum. But the membrane would be subjected to a considerable pressure, which would tend to make it rearrange its particles in such a

[1] Possibly this is the case in the *Fundulus* egg.

fashion that the volume enclosed within it might be lessened. Whereas an extremely rigid membrane would resist such forces, one with only a certain degree of rigidity would yield (in the case of sufficient pressure), and exosmosis would be possible. Thus osmosis in an enclosed system depends, to some extent at least, on the rigidity of the confining membrane. These conclusions apply in some measure to the sea-urchin egg, for the vitelline membrane possesses a slight degree of rigidity. Most salts in hypertonic solution cause the membrane to absorb water and swell, the gel becomes less stiff, and the particles of the membrane can more readily rearrange themselves. Thus shrinkage of the egg is favored. We should in fact expect that hypertonic solutions of salts which cause membrane swelling would be more effective in causing shrinkage than those which do not.

Direct evidence of this fact is difficult to obtain without introducing complications. One might compare the shrinkage of the egg in two solutions of equal osmotic strength, one of which causes membrane swelling and the other of which does not. But we have no means of knowing when two solutions are of equal osmotic power. Vant Hoff's law does not apply with sufficient accuracy to warrant its use,[1] and even if two solutions could be obtained which were isosmotic towards a given membrane, they would not necessarily be isosmotic towards the plasma-membrane of the *Arbacia* egg, which is very probably only partially semipermeable. Fortunately there is a way out of the difficulty. I found that when 2.5 *N* NaCl was added to sea-water in the proportion of 8 parts by volume of the former, to 50 of the latter, the resulting hypertonic solution would usually cause membrane swelling when freshly prepared, but would in large measure lose this power after it had stood for some time. Thus one can obtain two solutions of identical strength, one of which produces a softening effect on the membrane, the other lacking this effect. A number of experiments showed that in every case the eggs shrank more in the solution which caused membrane swelling than in the solution which left the membrane with its original rigidity. Two sample experiments are recorded here. To save time the term "NaCl

[1] *Cf.* Findlay, "Osmotic Pressure," London, 1913 (Chapt. IV.).

hypertonic sea-water" is used to designate 50 parts (by volume) of sea-water plus 8 parts of 2.5 *N* NaCl.

August 21. Two small stender dishes, *A* and *B*, were used. *A* contained 29 c.c. of NaCl hypertonic sea-water made up on August 18, *B* contained 29 c.c. of NaCl hypertonic sea-water freshly prepared. At 10:04 A.M., 5 drops of egg suspension were added to *A*, and a similar amount to *B*.

The following measurements of egg diameters were made at the times indicated. In making these measurements a Spencer movable scale micrometer was used. It was not found possible to obtain any very great accuracy in the use of this instrument. If the eggs are not subjected to pressure of the coverslip, they tend to move their position slightly. After various attempts I reached the conclusion that the use of the movable scale was inadvisable, especially as the usual difficulties of focusing made very accurate measurements out of the question. Accordingly the measurements were made with the scale stationary. No great claim for accuracy is therefore made, but the error is not over one micron. Fortunately the difference between the diameters of the two sets of eggs is markedly greater than the experimental error of the method. The measurements were made at a magnification of about 650 diameters.

Diameters of Eggs in *A*.

70 μ	×	70.5 μ	at	10.25 A.M.
69	×	71	at	10.28 A.M.
69	×	70	at	10.29 A.M.
67.5	×	69	at	10.30 A.M.
69	×	69	at	10.51 A.M.
68	×	71	at	10.53 A.M.
68	×	71	at	10.55 A.M.
69	×	69	at	10.57 A.M.
68	×	69	at	10.58 A.M.

Average 69.2 μ.

Diameters of Eggs in *B*.

66 μ	×	66 μ	at	10.32 A.M.
67	×	68	at about	10.35 A.M.
66	×	67	at about	10.35 A.M.
66	×	66	at about	10.35 A.M.
66	×	66	at	11.15 A.M.
70	×	70.5	at	11.17 A.M.

The above egg showed a completely unswollen membrane, and so should be excluded from the experiment.

63.5	×	65	at about	11.20 A.M.
67	×	67.5	at	11.24 A.M.

Average 66.2 μ (egg with unswollen membrane excluded).

The eggs used in the above experiment were all from a single female. The normal untreated eggs of this female were practically spherical and measured 74.5μ, 75μ, 75μ, 74μ, 75μ, 75μ, 75μ, 75μ, 75.5μ, 75μ. They thus possessed an average diameter of 75μ and were practically all of the same size. The experiment shows that the eggs in *B* with swollen membranes shrank more than did the eggs in *A* with unswollen membranes.[1] The average decrease in diameter of the former was about 9μ, of the latter about 6μ. The difference was also constant, for the largest egg with swollen membrane was smaller than the smallest egg with unswollen membrane.

August 23. Two small stender dishes were used. *A* contained 29 c.c. of "NaCl hypertonic sea-water," which had been made up on August 18 (at 11:30 P.M.). *B* contained 25 c.c. of sea-water. At 9.55 A.M., 4 drops of an egg suspension were dropped into *A* and the same amount in *B*, and then 4 c.c. 2.5 *N* NaCl were added to *B*, so that this solution became "NaCl hypertonic sea-water." The diameter of the eggs was determined as in the previous experiment. At 10:25 it was noticed that some of the eggs in *A* were beginning to acquire swollen membranes, so that only a few measurements were made after this time, and only those eggs with unswollen membranes were selected.

Diameter of Eggs in *A*.

65 μ	×	65 μ	at	10.12 A.M.
65	×	66	at	10.13 A.M.
67.5	×	70	at	10.14 A.M.
65	×	70	at	10.15 A.M.
65	×	68	at	10.16 A.M.
63.5	×	70.5	at	10.17 A.M.
65	×	66	at	10.18 A.M.

[1] This result was obtained in spite of the fact that membrane swelling always tends to produce an *increase* in egg volume. Whenever membrane swelling occurs in isotonic solutions, the egg rapidly increases its volume, it cytolyzes. The cause of this cytolysis resulting from membrane swelling will be considered later.

Diameter of Eggs in *A*.

65	×	68	at	10.19 A.M.
65	×	65	at	10.20 A.M.
66	×	66	at	10.26 A.M.
66	×	69	at	10.30 A.M.
66	×	68	at	10.31 A.M.
66	×	67	at	10.32 A.M.

Average 66.5 μ.

Diameter of Eggs in *B*.

63 μ	×	63.5 μ	at	10.04 A.M.
63	×	63	at	10.05 A.M.
64	×	65	at	10.06 A.M.
63	×	64	at	10.06 A.M.
64	×	65	at	10.07 A.M.
62	×	65	at	10.08 A.M.
63	×	63.5	at	10.08 A.M.
62	×	62	at	10.09 A.M.
63	×	63.5	at	10.10 A.M.
63.5	×	64	at	10.11 A.M.
64	×	65	at	10.34 A.M.
63.5	×	63.5	at	10.35 A.M.
63.5	×	66	at	10.36 A.M.
63.5	×	65	at	10.37 A.M.
63	×	63.5	at	10.38 A.M.
62	×	66	at	10.39 A.M.
64	×	64	at	10.40 A.M.
63.5	×	65	at	10.41 A.M.
63.5	×	64	at	10.41 A.M.
63.5	×	65	at	10.42 A.M.

Average 63.7 μ.

In this experiment also only eggs from a single female were used. The untreated eggs measured 72μ x 73μ, 72μ x 73, 71μ x 73μ, 71.5μ x 74μ, 71.5μ x 73μ. Considering the eggs as spherical, their average diameter was 72.4μ. Thus on the average, the diameter of the eggs in *A* decreased 5.9μ, whereas the diameter of the eggs in *B* decreased 8.7μ. Thus exosmosis was much more pronounced in the solution which caused membrane swelling than in a solution of equivalent concentration in which this effect was lacking.

II. Cortical Changes.

A. Membrane Elevation and Membrane Swelling.

When the sea-urchin sperm comes in contact with the egg, almost immediately the vitelline membrane is lifted away from

the egg surface. This is the well-known process of membrane elevation, or, as it is usually spoken of in this country, membrane formation. During elevation, the membrane under normal conditions does not undergo any evident increase in thickness. The inner border of the vitelline membrane is often not very plainly visible. In order therefore to estimate the thickness of the membrane after elevation, it is convenient to compress the eggs gently. The egg then becomes pushed out against the vitelline membrane, in some directions at least, and the distance between its outer border (*i. e.*, the hyaline layer, see below) and the outer boundary of the vitelline membrane is a measure of the greatest possible thickness of the membrane. Under such conditions, high power examination showed that the thickness of the elevated membrane is approximately the same as that of the unelevated membrane. Quantitative measurements were not found to be practicable.

Moreover, after elevation the membrane still retains the same chemical properties that distinguished it before fertilization. In dilute HCl it swells rapidly, and soon becomes sticky. NaI also induces the elevated membrane to swell, much as it did before fertilization.

After the vitelline membrane has been lifted from the egg surface, a new membrane appears around the cytoplasm. This structure has received an unusually large number of names; of these I shall use the term "hyaline layer." It seems reasonable to conclude that its formation depends on the same precipitation reaction which produces the membrane about egg fragments, and which is probably also responsible for the vitelline membrane. This conclusion is supported by the fact that it shows semipermeable properties.

The process of membrane swelling has often been confused with that of membrane elevation. This is perhaps due to the ambiguity of the term membrane formation. It has been the custom to apply the term whenever the observer notices something at the egg surface which he did not see at the beginning of the experiment. But membrane swelling and membrane elevation are two very different processes and are usually easily distinguishable under the microscope. The elevated

membrane appears as a thin membrane at some distance from the egg surface, which is now bounded by the above-mentioned hyaline layer. On the other hand, the swollen membrane appears as a homogeneous layer surroundng the egg, a layer in which neither the inner boundary of the vitelline membrane, nor the outer boundary of the hyaline layer, make their appearance. In addition to the purely morphological differences, there are other distinguishing features. Among these may be mentioned the fact that swollen membranes are always sticky, and as a result, eggs with such membranes tend to agglutinate. Normal elevated membranes are never sticky. Another criterion depends on the fact that elevated membranes collapse when placed in a solution of egg albumen (or other colloid).[1] Swollen membranes are of course unable to collapse.

B. Permeability Changes in the Vitelline Membrane.

The elevated membrane is known to be readily permeable to electrolytes.[2] Hence, since it offers considerable resistance to their passage before elevation, it must undergo a change in permeability at some stage in the process. An attempt was made to determine if this increase in permeability took place before or after membrane elevation. In the first case, it might be considered as causally related to the process, and R. Lillie has in fact suggested that the cause of membrane elevation is an increased permeability of the "plasma-membrane." Experiments, however, have shown that *the increase in permeability follows rather than precedes membrane elevation.* Immediately after elevation, the membrane is still more or less impermeable to electrolytes. The following experiments show this to be the case:

August 21, 1913. Eggs from a single female were washed twice and then gathered into about 10 c.c. of sea-water at the bottom of a small beaker. Four or five drops of diluted sperm were then added, and the beaker shaken. At intervals of 1, 2, 3, 4, 5 minutes after insemination, the eggs were re-

[1] It is only some few minutes after elevation that this collapse can be produced by a colloid. *Cf.* p. 162.

[2] *Cf.* Loeb, "Artificial Parthenogenesis and Fertilization," p. 208.

moved with a pipette and dropped into Syracuse dishes filled with 2 *M* $MgCl_2$. Just two minutes after insemination, a necessarily hasty examination of the eggs in the beaker showed that all the eggs had well-elevated membranes at this time. On the other hand, eggs removed from the beaker at the same time and placed in 2 *M* $MgCl_2$, upon later examination, showed no signs of an elevated membrane, and the membrane had evidently been pushed back against the egg. In the following table, the fractions in the second column indicate the proportion of eggs which showed the membrane elevated in the various Syracuse dishes. In each case, the numerator denotes the number of eggs with membranes elevated, the denominator the total number of eggs counted.

Minutes after Insemination Before Transfer to 2 *M* $MgCl_2$.	Membranes Elevated (Free from Egg).
1	0/50
2	0/50
3	1/50
4	30/50
5	49/50

In the case of the eggs transferred to the $MgCl_2$ solution three minutes after insemination, some of the membranes were not completely collapsed, but one, two, or even several small globular expansions could be detected at the egg surface.

This experiment was repeated on August 22, 1913, with almost identical results. In this case the eggs were transferred to 2 *M* $MgCl_2$ at intervals of 2, 3, 4 minutes after insemination. At $1\frac{1}{2}$ minutes after insemination hasty observation showed all the eggs to have well-elevated membranes. Thus the eggs were placed in the magnesium chloride solution after membrane elevation had occurred. Nevertheless, as the following table shows, the eggs removed to $MgCl_2$ two and three minutes after insemination, showed no membranes free from the egg.

After 2 minutes, 0/50 with membranes free from egg.
3 1/50
4 13/50

These experiments were also confirmed in the summer of 1914. I have interpreted the results as indicating that after elevation the membrane still remains impermeable to $MgCl_2$

(or as would no doubt be a more exact expression of fact, the membrane still offers sufficient resistance to the passage of $MgCl_2$ so that this salt exerts osmotic pressure against it). Indeed after being collapsed by the $MgCl_2$, the previously elevated membrane behaves as a semipermeable membrane and shrinks with the egg.

That the membrane is still "semipermeable" shortly after elevation is also shown by another series of experiments. It was found that the well-known collapse of the elevated membrane in solutions of egg albumen, did not take place immediately after elevation.

In an experiment of July 1, 1914, eggs were inseminated in a Syracuse dish at 9:43 A.M. Two drops of egg suspension were then placed in various Syracuse dishes containing 10 c.c. of 1 per cent. egg albumen[1] in sea-water, at intervals of 1, 3, 6, $12\frac{1}{2}$ minutes after insemination. In the case of the eggs placed in the albumen solution 1 and 3 minutes after insemination, the membrane was well elevated and had suffered no collapse. On the other hand, when the eggs which had been placed in the albumen solution 6 and $12\frac{1}{2}$ minutes after insemination were examined, their membranes were found to have been bent back and collapsed. This experiment was repeated a number of times, similar results being obtained in each case.

These surprising results find an easy explanation on the ground that the membrane is still partially permeable shortly after elevation. Because of this semipermeability it is subjected to the pressure of the electrolytes contained within it. Only when the membrane loses its semipermeable properties does this pressure cease and only then can the albumen cause its collapse. The albumen seems to exert a protective effect on the membrane and to prevent increase of permeability. Thus, when eggs were placed in albumen 1 minute after insemination, their membranes were found to retain their semipermeability and they collapsed upon being placed into 2 *M* $MgCl_2$ 14 minutes after insemination.

From these experiments we can, I think, conclude that increased permeability follows upon, rather than precedes, mem-

[1] Kahlbaum's crystallized egg albumen was used. The solution was filtered.

brane elevation. At a later point, in discussing what I believe to be the true explanation of membrane elevation, I shall endeavor to point out a reason for this sequence.

C. Theories of Membrane Elevation.

The problem of membrane elevation is quite distinct from that of segmentation, which is the central theme of the study of artificial parthenogenesis. No method of producing membrane elevation ordinarily results in more than one or two per cent. of segmentations, and many methods usually result in no segmentations whatsoever (*e. g.*, chloroform, urethane). Moreover, some of the best methods for producing segmentation do not involve membrane elevation: thus hypertonic sea-water never results in an elevation of the membrane.

Of late Loeb has been of the opinion that membrane "formation," involves the swelling of some substance at or near the egg surface. To quote from his book[1] "a colloidal substance which lies below the surface layer of the unfertilized egg or is secreted from the egg, suddenly swells by absorption of sea-water. In the typical case of membrane formation this swelling results finally in a complete liquefaction of the colloid. In other cases the swelling is less complete and the formation of a gelatinous film results." Loeb thus regards the process of membrane formation as due to the swelling of a colloid, concerning the position of which he is not quite clear. On page 213 of the same book he says it is in the cortical layer. Finally, if the egg is left too long a time in a solution which causes membrane formaton, not only a colloid at the periphery, but colloids throughout the egg swell, and cytolysis results.

In the discussion of Loeb's theory, I shall assume that he uses the word "swell" in its colloid-chemical sense, *i. e.*, to denote the absorption of a liquid (in this case water) by a gel. Of course any less specific use of the term would rob the theory of all theoretical significance. Briefly, the objections to the swelling theory, as upheld by Loeb, are:

1. All the reagents which cause membrane elevation can scarcely induce the swelling of any one colloid. Loeb claims

[1] *Loc. cit.*, p. 210.

that some of the "membrane-forming substances" cause the egg membrane of the Mollusk *Lottia* to swell, but he admits that this is not true of all of them. Some seem to cause swelling and final liquefaction of the chorion or jelly of the sea-urchin egg, but here again all are not effective, and I can suggest chloroform as an exception. In fact it must be a strange colloid which can be made to absorb water by such reagents as distilled water, alcohol, chloroform, toluol, picric acid.

2. It is difficult to conceive of the location of the swelling colloid. The egg has been shown to consist essentially of a more or less rigid membrane surrounding a mass of fluid contents. Evidently the latter can not swell, as only gels possess this property. It is also demonstrable that the outer vitelline membrane itself does not swell in the case of true membrane elevation, for it can scarcely be doubted that the vitelline membrane undergoes an increase rather than a decrease of rigidity after elevation. And swelling is always correlated with a decrease in rigidity on the part of the gel. It is difficult to understand how Loeb seeks to explain by swelling what he regards as the *formation* of a more or less rigid membrane.

3. Cytolysis results not in colloidal swelling and liquefaction, but in coagulation. Loeb considers membrane elevation and cytolysis as due to the same processes. He says:[1] "Substances like benzol, saponin, etc., can cause both membrane formation and cytolysis. The first of the two is produced when they have time to affect only the surface of the egg; cytolysis is produced when their effect extends to the deeper layers of the egg . . . the greater the fraction of the egg which comes under the effect of the membrane-forming reagents, the greater the amount of colloid that must be liquefied." Loeb thus states explicitly that the membrane-forming reagents "liquefy" the colloids of the eggs, and that this effect in the case of cytolysis extends into the interior. Now it is a fact that the membrane-forming reagents, far from producing liquefaction, have an exactly opposite effect upon the colloids of the *Arbacia* egg. Instead of transforming a solid mass to a more fluid state, what they really do is exert a solidifying or rather a coagulating effect

[1] *Loc. cit.*, p. 213.

upon the egg colloids. The actual evidence in support of this statement will be given at a later point (p. 196).

In the first paper of this series, after showing that all substances which produce membrane elevation cause a lowering of surface tension, I proposed a theory of membrane-elevation based on a simple consideration of the forces in equilibrium at the vitelline membrane. At the time I was not yet aware of the semipermeable nature of this membrane, and hence in discussing the various forces acting upon it, I did not consider osmotic forces as being involved. The theory therefore requires slight modification in the light of this new fact. Let

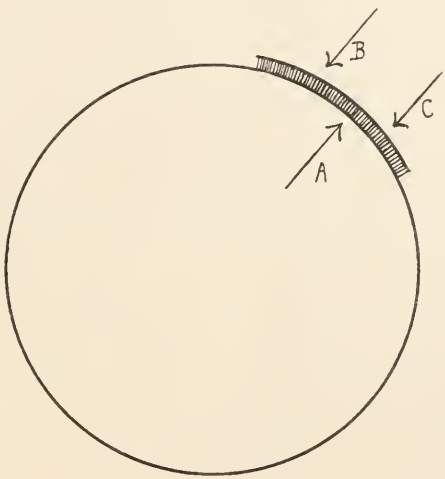

FIG. 1.

us suppose in Fig. 1 that the shaded area is the vitelline membrane. The arrows represent the forces acting upon it. Arrow *A* directed outward indicates the outward force due to the osmotic pressure of the dissolved substances within the membrane. Were gels present in the egg we should also have to add the swelling pressure of these. Directed inward are two arrows, arrow *B* is the force due to the osmotic pressure of the salts outside the membrane, arrow *C* is the radial component of the surface tension of the membrane. As the membrane is slightly thicker than twice the range of molecular action, it is really composed of two surfaces of surface tension, *i. e.*, an outer surface of contact with the sea-water and an inner surface of

contact with the egg contents. Both surfaces are solid-liquid surfaces, and as such no doubt possess a high surface tension.[1] The arrow *C* represents the sum of the radial components of the tension of both inner and outer surfaces of the membrane. Suppose now that a substance which lowers the surface tension of water, is added to some eggs in sea-water. By what is known as the Gibbs-Thomson Law it will tend to accumulate at the surfaces of the vitelline membrane. This will result in a lowering of the surface tension of the membrane.[2] As a result equilibrium no longer exists, the force pushing outward is now stronger and the membrane is lifted away from the egg surface. Most of the egg proteins do not follow the membrane as it is lifted away, but remain in their original position. This is due to the fact that they diffuse much less readily than the salts, which can be thought of as pushing out the membrane. Around the mass of egg proteins a membrane is rapidly formed. As previously pointed out, this "hyaline layer" is to be regarded as similar to the precipitation membranes formed on egg fragments.

As a result of elevation, the vitelline membrane is no longer

[1] The surface tension of a solid-liquid surface has never been accurately determined, but there are various reasons for considering it the seat of an exceptionally high tension. For the similar case of a solid-gaseous surface Freundlich ("Kapillarchemie," p. 90) following Quincke states that "die Oberflächenspannung flüssig-gasformig steigt allgemein mit sinkender Temperatur. Wenn nun die Flüssigkeit stetig in einen amorph-festen Korper—eine Flüssigkeit mit sehr groszer innerer Reibung—übergeht, musz man auch annehmen, das die Oberflächenspannung bestehen bleibt, ja das sie zunehmend gröszere Werte erhält wenn sie sich auch wegen der groszen Zähigkeit nicht äuszern kann." With the aid of a formula derived by Wilh. Ostwald, Freundlich calculates the tension of solid-liquid surface $BaSO_4$-water to be several thousand dynes per cm., an enormous value. In his wonderful treatment of capillarity Gibbs devotes a long section to the discussion of solid-liquid surfaces (Gibbs, Collected Papers, I., 314–331), and derives various equations for them. In discussing the surfaces of the membranes, I have for the sake of simplicity not considered the presence of the jelly of chorion, which surrounds the egg. This is so diffuse a gel that it no doubt has little if any effect. In fact, after its removal (by shaking), the eggs behave just as they did before.

[2] Gibbs, *loc. cit.*, p. 274: "Now the potential of a substance which forms a very small part of a homogeneous mass certainly increases, and probably very rapidly, as the proportion of that component is increased. (See (171) and (217).) The pressure, temperature, and the other potentials, will not be sensibly affected (see (98)). But the effect on the tension of this increase on the potential will be proportional to the surface-density, and will be to diminish the tension when the surface-density is positive (see (508))." The numbers refer to equations. When a substance accumulates at a surface, its surface density is by definition positive.

in contact with the egg cytoplasm. It is, accordingly, now exposed on both of its faces to a solution which has the power of coagulating it. It might be expected that the process of gelatinization or coagulation be carried a step farther, and that the membrane become more rigid. This increased coagulation, if I may so speak of the process,[1] results, as is typical for such cases, in an increase of refractive index, and the optical image of the membrane becomes more clearly defined. It may also result in an increased permeability of the membrane, for Quincke ('77) claims that precipitation membranes lose their semipermeable properties on becoming rigid.

On the basis of the above theory, it is evident that when the surface tension of the membrane drops below a certain limiting value, elevation occurs. The determination of this value is at present impossible. It might be thought that the determination of the surface tension of the various membrane-elevating solutions at their surface of contact with air, would throw some light upon the matter.[2] But the lowering effect of a substance on a solid-liquid (or liquid-liquid) surface tension can not be measured by the effect the same substace produces on a gas-liquid surface. The chief reasons for this are: (1) Adsorption of added substances plays a very decided rôle in the case of the solid-liquid surface, different solids of course showing different degrees of selective adsorption, (2) A solid-liquid surface is not subject to the action of evaporation, as is a gas-liquid surface. This is of especial importance if the substance which lowers surface tension is volatile. In discussing films like those of bubbles, Gibbs[3] says: "But when a component which greatly diminishes the tension of the film although forming but a small fraction of its mass (therefore existing in excess at the surface), is volatile, the effect of evaporation and condensation may be considerable, even when the mean value of the potential for that component is the same in the film as in the surrounding atmosphere."[4] Thus chloroform in water is quite effective

[1] It might also be designated as "loss of water."

[2] Czapek ('11) has thus endeavored to draw conclusions as to the surface tension of the plasma membrane of plant cells by measurements of air-liquid surface tensions.

[3] *L. c.*, p. 310.

[4] A condition which would at least be approximated if the film were in equilibrium with its vapor in an enclosed chamber.

for membrane elevation, but such a solution of chloroform has a surface tension against air just slightly below that of pure water.[1] The great volatility of chloroform explains its inability to lower markedly the tension at the water-air surface; it is unable to accumulate in the surface film. But it can readily accumulate at a solid-liquid surface and here it no doubt produces a marked lowering of surface tension. It follows that, although by a measurement of the air-water surface tension we can determine qualitatively whether a substance lowers the surface tension of the membrane or not, any truly quantitative measurements are impossible.

In order to show qualitatively that a substance lowers the surface tension of the membrane, it is only necessary to make certain that its solution in water has a lower surface tension than the pure solvent. The theoretical basis for this assertion has already been pointed out. There is also another case to be considered. Some substances exert a liquefying effect upon the vitelline membrane. The liquefaction of a gel no doubt results in a considerable lowering of its surface tension.[2] Ordinarily, however, the liquefying effect is a slow one, and in such cases, membrane elevation does not occur. For if the surface tension of the membrane is lowered only very slowly, the egg proteins have time to follow the membrane as it is pulled outwards. The result is an increase in egg diameter; the egg cytolyzes. Thus when the surface tension of the vitelline membrane is slowly lowered, cytolysis follows directly.

On the other hand, when it is rapidly lowered membrane elevation takes place first and cytolysis follows after a short interval. After the vitelline membrane is elevated, it loses its semipermeability and the hyaline layer takes its place as the plasma-membrane of the egg cell (cf. p. 159). The same forces act on this membrane as were found to act on the vitelline membrane prior to its elevation. Hence when its surface tension is lowered, since the osmotic pressure within the egg is no longer completely counterbalanced, the hyaline layer tends to become pushed outward. But the hyaline layer appears to be more closely adherent to the egg proteins[3] so that they are pulled out

[1] Czapek ('11), p. 35.

[2] *Cf.* note p. 166.

[3] Probably because of a slight cortical coagulation.

with it and the entire egg increases in diameter. According to this view cytolysis (when not directly an osmotic phenomenon) is in every case due to a lowering of the surface tension of the plasma membrane. This produces an influx of water into the cell.

Various other explanations of cytolysis have been attempted. It has been regarded as due to the swelling of the egg proteins. This is an incorrect assumption, as I have already pointed out that the egg proteins, instead of becoming swollen, are coagulated in the cytolyzed egg (see pp. 195–198). It might also be considered as the result of a change in the plasma-membrane, of such a sort that this membrane would become readily permeable for salts though still impermeable for colloids. The unopposed osmotic pressure of the egg colloids would then push out the plasma membrane and the egg would increase its diameter. But such an explanation can not hold generally, for membrane-swelling although it produces cytolysis, does not render the plasma-membrane readily permeable to salts. Indeed in hypertonic salt solutions, eggs with swollen membranes shrink even more than those with unswollen membranes (see pp. 155–158). This is sufficient proof that the swollen membrane retains its semipermeability.

In an earlier paper it was pointed out that all substances which had been described as producing membrane elevation (as well as some other newly discovered ones) did actually lower surface tension in aqueous solution. In a few substances, however, this lowering could apparently only be a very slight one. These cases have been reinvestigated, and they have been found to involve membrane swelling rather than membrane elevation.

When membrane swelling is rapid, it is possible that the lowered surface tension so produced should be followed by membrane elevation if the eggs are immediately returned to sea-water. One such case was indeed observed, but the observation was made at the close of the season, and further study of this point is necessary.

D. Cortical Action of Heat and of Various Chemicals.

Heat.—Although heat has been described as producing artificial parthenogenesis in the sea-urchin egg (McClendon, '10), I know of no description of membrane elevation as a result of heat treatment. In several experiments, I was unsuccessful in producing membrane elevation in this fashion. In experiment *a*, eggs were dropped into sea-water which had been heated to 36.5 degrees, and were exposed 1, 2, 3, 4 minutes. In experiment *b*, the eggs were dropped into sea-water heated to 35 de-

grees, and were exposed 2, 4, 6, 8 minutes. In experiment *c*, eggs were dropped into sea-water heated to 37.5 degrees and kept at this temperature on a waterbath; the eggs were exposed $3\frac{1}{4}$ minutes. In no case could any membrane elevation be observed, but the vitelline membrane did appear to be slightly swollen after the heat treatment.

Alkalis.—If alkali is added to sea-water, the resultant precipitation of magnesium salts tend to convert the sea-water into a solution containing little else than sodium salts. No doubt this fact alone is of significance. It is probable, however, that there is a more specific action of the alkali. As was shown in a previous discussion (p. 151), alkali apparently is able to liquefy the vitelline membrane. It is doubtful if any observer has actually obtained membrane elevation with NaOH or KOH. Certainly it does not occur when the eggs are left in the alkaline solution.

Butyric Acid.—This famous method is one of the best for artificial membrane elevation. Loeb used it originally on *Strongylocentrotus* eggs, employing a concentration of 50 c.c. sea-water plus 2.8 c.c. *N*/10 butyric acid and exposing the eggs $1\frac{1}{2}$–3 minutes. In his recent book, he applies the method to *Arbacia* eggs reducing the concentration of acid slightly (50 c.c. sea-water plus 2.0 c.c. *n*/10 butyric), but retaining the same time of exposure. He says that the eggs do not form a conspicuous fertilization membrane, but only a "fine gelatinous layer which was not easily visible." This is evidently a membrane swelling and not an elevated membrane. For the benefit of workers in the field, I mention a slight modification in the method, by which true elevated membranes may be obtained for *Arbacia* eggs. Instead of $1\frac{1}{2}$–3 minute exposures, a $\frac{1}{2}$ minute exposure was found very effective, and over ninety per cent. of elevated membrane could regularly be obtained if the eggs were well washed and in good condition. The concentration used was the original one of Loeb's, 50 c.c. sea-water plus 2.8 c.c. *N*/10 butyric; slight variations are however immaterial. Although eggs exposed 1 minute often showed membrane elevation, no elevation was found to occur on longer exposure. Instead the membrane in every case appeared swollen, the amount of swelling increasing with the length of exposure.

Inorganic Salts.—That isotonic solutions of various salts cause membrane "formation" was discovered by R. Lillie ('10). The effectiveness of the various salts was found to correspond with their order in the lyotropic series, *i. e.*, those salts which are most effective in producing swelling of protein gels (in alkaline solution) were also most effective in inducing membrane "formation." The reason for this correspondence becomes apparent, when we consider that the membrane "formation" produced by isotonic salt solutions is nothing other than a membrane swelling. The membranes resulting from salt treatment are sticky, they do not collapse in the presence of a colloid (cf. p. 160).

The concentration of a salt is an important factor in determining whether swelling shall take place, and how pronounced the swelling shall be. Let us consider as an example the case of NaCl. Seven solutions of NaCl were prepared by adding distilled water to a 2.5 normal solution.

Thus Solution *A* contained 50 c.c. $2\frac{1}{2}$ *M* NaCl plus 0 c.c. distilled water.

B	40	10
C	30	20
D	20	30
E	10	40
F	5	45
G	$2\frac{1}{2}$	47.5

Eggs were placed in all these solutions at 3:30 P.M. By 3:39 eggs in *A* showed a membrane swollen to 2.5μ in diameter. By 4:50 it had reached approximately 5μ.

As for the eggs placed in *B*, the membrane swelled at least as rapidly as in the first case.

Membranes on eggs in *C* and *D* also swelled rapidly, but in no case did eggs in *E*, *F*, *G* show any membrane swelling. Thus a certain concentration is necessary for swelling to occur.

There appears to be at least two factors involved in this effect of concentration. In the first place, the swelling action of the salt no doubt increases with the concentration. But it is probable that the hypertonicity of the solution may also play a rôle.[1]

[1] The explanation of such an effect might be as follows: When water is extracted from the cell, there is a tendency for vacuum production and consequent negative pressure (release of pressure) on the particles of the membrane. But we know

Sensitization with Strontium Chloride.—The process of sensitization was originally used by Loeb, later by Robertson, to induce the "formation" of a membrane in eggs exposed either to blood sera or to various tissue extracts, all dissoved in what was essentially a NaCl solution isotonic with sea-water. The method employed consists either of adding 3/8 *M* $SrCl_2$ directly to the serum, or of placing the eggs first into the 3/8 *M* $SrCl_2$ for several minutes, then into the serum. In the first paper of this series I urged that $SrCl_2$ caused a precipitation of sulphates and that much of the effect of sensitization was no doubt due to this action. In the summer of 1913, I was able to prove the truth of this statement. On July 31, 10 c.c. of 3/8 *M* $SrCl_2$ was added to 90 c.c. of sea-water. The voluminous precipitate of $SrSO_4$ was allowed to settle and filtered off, but the precipitate still continued to settle from the filtrate. When eggs were placed into this filtrate membrane swelling occurred, and this was followed by cytolysis. In another experiment, the eggs were placed first into 3/8 *M* $SrCl_2$ and then into a 0.55 *M* NaCl solution. Used alone, the NaCl solution did not cause membrane swelling when two drops of a thick egg suspension were placed into 75 c.c. of it. But when several drops of egg suspension were placed into 3/8 *M* $SrCl_2$, and then after five minutes, 2 drops of liquid containing eggs were taken from the $SrCl_2$ solution and placed into the NaCl solution, membrane swelling could be observed to take place in the eggs so transferred. The vitelline membrane could be seen slowly to increase in thickness, so that after about half an hour a good proportion of the eggs were surrounded by a transparent outer layer which bore a close resemblance to an elevated membrane, except for the fact that the inner boundary of such a membrane was absent. The membranes produced as a result of the "sensitization" process did not collapse in the presence of a colloid. They were also sticky, and as a result the eggs tended to agglutinate. Such an agglutination of eggs with swollen membranes has no doubt led Robertson ('12) to the view that "fertilization and agglutination are similar phenomena."

from Le Chatelier's theorem that whenever the pressure on a system in equilibrium is diminished, a change or reaction ensues which is accompanied by increase of volume. Hence the swelling.

These facts enable us to interpret many of the results of Loeb and Robertson on a truly chemical basis, and without the aid of a hypothetical lysin. But there is also another factor to be considered in the analysis of the action of sera, and tissue extracts. It is the fact that many proteins are probably able to effect a swelling of the vitelline membrane. Such an action is at least true for egg albumen. The eggs of 2 females were washed twice and pipetted into 100 c.c. of 1 per cent. egg albumen in sea-water. Before ten minutes had elapsed the membranes on most but not all of the eggs had swollen considerably. The swelling action of albumen seems to vary considerably, sometimes being almost negligible. It is, I think, to be regarded as a phenomenon akin to peptonization, the albumen playing the part of a protective colloid.

KCN.—The action of potassium cyanide is rather difficult to explain on basis of the surface-tension theory. Although KCN alone, in the dilutions used, never caused elevation of the membrane, if the solution was made hypertonic the membrane did separate from the egg. Thus on July 31, 1914, membrane elevation did not occur in 25 c.c. of sea-water plus 1 c.c. of 1/20 per cent. KCN, but in a similar solution plus 4 c.c. 2.5 *M* NaCl, the vitelline membrane in practically every case became lifted away from the egg surface. How can this observation, which was repeated several times, be interpreted on the basis of the surface-tension theory? As is well known, KCN hydrolizes readily, so that KOH and HCN are always present in a solution of the cyanide. Probably HCN plays the most important part, for it is a gas, and hence its surface tension is practically zero. Thus it no doubt lowers the surface tension of the vitelline membrane (cf. p. 166). But membrane elevation does not result, because of another effect of the cyanide. In the first paper of this series, it was pointed out that even if a substance lowered surface tension, it would not produce membrane elevation if it increased too greatly the modulus of elasticity of the vitelline membrane, for in that case the toughened membrane would be incapable of stretching. There is evidence that KCN actually does increase the modulus of elasticity. In the next section it will be shown that the presence of KCN retards membrane swelling, and such anti-swelling action is a general characteristic of all

substances which increase the modulus of elasticity.[1] As a result of the stiffening action of KCN, the membrane resists elevation, for the elevating force is not sufficient to stretch it. But in a hypertonic solution, as the egg shrinks, no stretching is necessary to separate the vitelline membrane from the egg, and the membrane is accordingly pulled away from the egg surface.

E. Inhibitors to Membrane Swelling.

It has been found that swelling of the vitelline membrane may be retarded or even inhibited in the presence of certain substances. Up to the present only two such inhibiting substances have been discovered. Of these, KCN inhibits the swelling of the membrane by salts, sea-urchin blood on the other hand inhibits acid swelling. But the inhibitor of salt action has no such effect on acid swelling, and sea-urchin blood instead of inhibiting swelling produced by salts, actually seems to favor it. These results, paradoxical as they appear at first sight, are really in direct line with recent findings in the field of colloid chemistry. There it has been shown that the salt and the acid swellings of gelatine must be essentially different processes, for the very salts which, by themselves, favor or even cause swelling, retard the swelling effect of an acid.

Although membrane swelling usually occurs in "NaCl hypertonic sea-water,"[2] in the presence of traces of KCN no such swelling will occur. This inhibiting effect of KCN has as yet not been found to be shared by any other substance, but so far only a few experiments in this direction have been made. Some of the experiments made during the summers of 1913 and 1914 are recorded below.

August 29, 1913. To 200 c.c. of sea-water was added 32 c.c. of 2.5 *M* NaCl and the resultant "NaCl hypertonic sea-water" was divided into four 50 c.c. portions, *A*, *B*, *C*, *D*. To *A* was added 0.5 c.c. 1 per cent. KCN, to *B* 0.5 c.c. *N*/10 NaOH, to *C*, 0.5 c.c. ether, and nothing was added to *D*, which several as a control.

Eggs were then placed in *A*, *B*, *C*, *D*, and it was found that although membrane swelling very evidently took place in *D*,

[1] Freundlich, "Kapillarchemie," p. 512.

[2] As previously stated, I use this term to indicate a solution of 50 parts (by volume) of sea-water plus 8 parts of $2\frac{1}{2}$ *M* NaCl.

no such swelling occurred in *A*. The effect was not due to the alkaline reaction of KCN, for the swelling of egg membranes in *B* showed that NaOH had no retarding effect on the process. Likewise the ether present in *C* appeared to have no inhibiting effect.

In other experiments KCN behaved similarly. In general it was found advisable to first place the eggs into sea-water which contained KCN and to add the 2.5 *M* NaCl later. Thus on August 29, 1914, it was found that some membrane swelling occurred when eggs were dropped into hypertonic sea-water to which KCN had already been added, but that this was entirely prevented when the eggs were first exposed to the KCN solution in sea-water, the 2.5 *M* NaCl being added later.

August 29, 1914. Fingerbowls *A*, *C*, *D*, were used. Into *A* were placed 50 c.c. sea-water plus 8 drops of egg suspension, and to this were added 8 c.c. of 2.5 *M* NaCl. Fingerbowl *C* contained 50 c.c. sea-water plus 1 c.c. 1/5 per cent. KCN, to this were added at 10:30½ A.M. 8 c.c. 2.5 *M* NaCl, and at 10:31 A.M. 8 drops of egg suspension were dropped into the cyanide containing "NaCl hypertonic sea-water." Fingerbowl *D* contained 49 c.c. of sea-water plus 1 c.c. 1/5 per cent. KCN. Several drops of egg suspension were added to this solution of cyanide in sea-water, at 10:40 A.M. At 11:28 A.M. (48 minutes later), 8 c.c. of 2.5 *M* NaCl were added to *D*.

On microscopical examination, it was found that pronounced membrane swelling had taken place in *A* (in the absence of KCN), swelling also occurred in *C*, but did not appear to be pronounced as that occurring in *A*. No membrane swelling at all could be observed in *D*, in which the eggs had been treated first with KCN before the concentrated NaCl solution had been added.

The above experiment indicates that the action of KCN in inhibiting membrane swelling produced by NaCl, is not the result of a reaction between the cyanide and the salt, but is due to an effect of the cyanide on the membrane. For if only a reaction between salt and cyanide was involved, there could be no advantage in first subjecting the eggs to the action of the cyanide alone.

Although KCN inhibits the membrane swelling effect of

NaCl, it does not appear to have the slightest retarding effect on membrane swelling when this is produced by an acid.

August 28, 1914. Fingerbowl *A* contained 50 c.c. sea-water plus 3 c.c. *N*/10 butyric acid. Fingerbowl *B* contained 49 c.c. sea-water plus 3 c.c. *N*/10 butyric acid plus 1 c.c. 1/10 per cent. KCN. When eggs were added to *A* and *B*, membrane swelling occurred in both.

September 3, 1914. Stender dish *A* contained some eggs in 25 c.c. of sea-water. To this was added 1 c.c. 1/5 per cent. KCN at 10:52 A.M. Then 2 c.c. *N*/10 butyric acid were added 29 minutes later (at 11:21).

Stender dish *B* contained eggs in 25 c.c. of sea-water, 2 c.c. of *N*/10 butyric acid were added at 11:22 A.M.

In both *A* and *B*, membrane swelling occurred and as a result the eggs in both cases stuck to each other and to the bottom of the dish. No difference could be observed between the two sets of eggs, and apparently the KCN has no effect on acid swelling of the membrane.

KCN is thus capable of inhibiting membrane swelling by NaCl, but it has apparently no effect when the swelling is produced by butyric acid. On the other hand, sea-urchin blood was found to retard or inhibit acid swelling.

June 22, 1914. A solution of butyric acid in sea-water was prepared by adding to 50 c.c. of sea-water, 2.5 c.c. of *N*/10 butyric acid. Approximately 5 c.c. of the resulting solution were placed in each of two Syracuse watch-crystals (*A* and *B*). To watch-crystal *A* were added 3 c.c. of filtered sea-urchin blood. (The blood was filtered after it had been allowed to "clot" by standing.) To watch-crystal *B*, 3 c.c. of sea-water were added. In *B*, membrane swelling and agglutination occurred, in *A* very little, if any, membrane swelling occurred, and there was no agglutination. The jelly was dissolved away from the eggs in *A*, but although the eggs were thus able to come into close contact, they would separate again, showing that they were not sticky, and that no membrane swelling had occurred. After a few hours, eggs in *B* had completely lost their color and appeared white to the naked eye, those in *A* appeared normal.

July 7, 1914. The above experiment was repeated. In this case an acid solution was made up by adding 5 c.c. *N*/10 butyric

acid to 50 c.c. of sea-water. Of this solution, 5 c.c. was put into each of two Syracuse watch-crystals *A* and *B*.

To *A* were added 5 c.c. of sea-water and five drops of egg suspension.

To *B* were added 5 c.c. of filtered blood (from several ♂s and ♀s) and then 5 drops of egg suspension.

The result of the experiment was that in *A* the vitelline membranes swelled in practically every case. A count of a hundred eggs gave ninety-nine eggs with swollen membranes and the single exception was doubtful. On the contrary, there was practically no membrane swelling in *B*, which contained blood in addition to the acid. A count gave, of a hundred eggs observed, only three with swollen membranes.

The inhibiting effect of blood upon acid swelling, unlike the effect of cyanide on salt swelling, may perhaps be the result of a direct action of the blood upon the acid. This is barely suggested by the fact that *N*/10 HCl produces a flocculent white precipitate when added to filtered sea-urchin blood. However in the above recorded experiments with butyric acid, no such precipitation could be observed.

Instead of exhibiting a retarding effect upon membrane swelling by salts, sea-urchin blood seemed to favor the process. This favorable effect was much more pronounced in some cases than in others, and in several experiments it was not readily observed. On July 28, 1914, membrane swelling was found to be much more rapid and pronounced in a solution of 20 c.c. sea-water plus 5 c.c. filtered blood (from ♀s) plus 4 c.c. 2.5 *M* NaCl, than in a similar solution without blood, *i. e.*, 25 c.c. sea-water plus 4 c.c. 2.5 *M* NaCl. It sometimes happens, and this appeared to be more frequent in 1914 than in 1913, that membrane swelling does not occur in "NaCl hypertonic sea-water." In such cases, it was found possible in several instances to produce a membrane swelling by the addition of blood. Thus on July 30, 1914, although there was no swelling in "NaCl hypertonic sea-water," when eggs of the same lot were placed into 5 c.c. blood plus 20 c.c. sea-water plus 4 c.c. 2.5 *M* NaCl membrane swelling did occur.

It might be reasoned that this action of blood is analogous to the cytolytic effect of sera foreign to the individual. But

in one case at least, blood of the same individual from which the eggs had been taken, was found to exert an accelerating effect upon membrane swelling in "NaCl hypertonic sea-water."

If it be true that blood retards acid swelling and favors salt swelling, this fact can be used in cases of doubt, to determine if a given type of membrane swelling is the result of the action of a salt or an acid.

F. Cortical Changes at Fertilization.

The central object of studies in artificial parthenogensis is to find an explanation of the processes occurring in normal fertilization. The fact that artificial membrane elevation is apparently always the result of a lowered tension of the vitelline membrane has of course led to the view that the spermatozoön also produces a lowered surface tension. There are two conceivable ways in which this could happen. In the first place the sperm might carry a substance which lowers surface tension directly. This is improbable, in view of the fact that it has not been possible to extract from sperm a membrane-elevating substance. It is more logical to suppose that the very act of penetrating on the part of the sperm lowers the tension.

If the tension of a stretched thread be lowered at one point, instantaneously the tension throughout the thread is lowered. Similarly if the tension of a spherical stretched film or membrane be lowered at one point, there will be a lowering of tension in every point of the film, for in order that equilibrium be established, the tension in every part of the spherical film must be equal. This equalization of tension is probably a rapid process, especially when not merely a point, but an appreciable area of the surface has its tension lowered.[1]

Thus the penetration of the sperm almost immediately produces a lowered tension in all parts of the vitelline membrane. In the sea-urchin egg, the sperm can not bore its way through the membrane mechanically, as it is not provided with a perforatorium. It is therefore probable that the sperm has a solvent action on the membrane upon coming in contact with

[1] However, in cases where a very thin film surrounds a large spherical mass as in air-bubbles the attainment of equilibrium between the parts of the film is much slower than the attainment of equilibrium between the film and the contiguous fluids. *Cf.* Gibbs, *l. c.*, p. 300.

it. The partial liquefaction or swelling of the vitelline membrane at the point of sperm entrance can be conceived of as serving two functions: (1) it enables the sperm to enter, (2) it lowers the surface tension of the membrane and thus produces membrane elevation.[1]

That the sperm actually does produce a substance capable of causing membrane swelling, can be demonstrated. It is not possible to observe the swelling produced by a single sperm. But if the eggs are placed into very concentrated sperm suspensions, the vitelline membrane can be seen to swell all around the egg. Such concentrated suspensions are obtained by allowing the sea-urchins to shed their sperm. As is well known, the shedding reaction is aroused when the oral part of the shell is cut away. If the "dry" sperm be diluted only very slightly, an enormous sperm concentration can be obtained. When eggs are mixed with sperm suspensions of such high concentration, each egg immediately becomes surrounded by a halo of wriggling sperm. Soon the vitelline membrane can be seen slowly to increase in thickness, it swells until it may reach a thickness of about 3 microns. That normal membrane elevation has not taken place can be shown by the fact that the swollen membranes thus produced do not collapse when the eggs are placed in a 1 per cent. or a 2 per cent. albumen solution (in sea-water). In a concentrated sperm suspension, each point on the vitelline membrane is a point of attack on the part of the spermatozoa, and the entire membrane becomes swollen.[2]

The concentration of sperm necessary to produce a complete

[1] It might be thought that puncture of the vitelline membrane, *e. g.*, by a needle, should produce elevation. But this is not necessarily the case, for the hole produced by a mechanical puncture of the membrane, if not immediately closed, would involve a loss of its semipermeable properties, and these on the basis of the theory (see pp. 165–166) are necessary for membrane elevation. A deep prick would also produce coagulation of the underlying cytoplasm, which would tend to prevent elevation.

[2] It might be asked why elevation of the membrane does not follow swelling produced by concentrated sperm suspensions, since this no doubt results in a rapid lowering of surface tension. The answer is clear. In a previous paper (Heilbrunn, '13) it was pointed out that membrane elevation never occurred when the egg or its cortex was coagulated. Now in concentrated sperm suspensions, it can be shown that a profound coagulation does take place. This was demonstrated by a method which has been developed for revealing the presence of coagulation within the egg. For details of this method see p. 192.

swelling of the membrane varies with the season. In the height of the season only quite concentrated sperm suspensions produce the phenomenon. But towards the end of August, as the season begins to wane, more and more dilute suspensions become effective, until at the very close of the season, it is difficult to secure true membrane elevation at all. Probably the fluid emitted with the sperm is then charged with the substance which causes the swelling.

In the preceding section it was shown that sea-urchin blood retards acid membrane swelling, but favors salt membrane swelling. This fact makes it possible to determine the general nature of the membrane swelling produced by sperm. One has only to observe the action of concentrated sperm suspensions in the presence of blood. If blood favors the swelling, this can be taken as evidence that the sperm action is similar to that of salts, if on the other hand it retards swelling, an acid is probably responsible. On July 7, 1914, 5 c.c. of sea-water plus 9 drops of egg suspension plus 5 drops of "dry" sperm were mixed in Syracuse watch-crystal *A*. Watch-crystal *B* contained 4 c.c. of blood (filtered from ♂s and ♀s) plus 9 drops of egg suspension plus approximately the same amount of sperm as did *A*. Both watch-crystals were shaken slightly to insure mixing. In *A*, the membranes swelled gradually; after 43 minutes they measured approximately 2μ. In *B*, on the contrary, no membrane swelling could be observed.

It might be objected that in the above experiment the blood had some effect which prevented intimate contact of sperm with egg. This objection is obviated by the following experiment. It was performed at the very close of the season, at a time when as previously pointed out, sperm suspensions have a much greater tendency to produce swelling. On September 1, 1914, considerable difficulty was experienced in procuring sperm. Ten males were cut open and allowed to shed (several others had been rejected as being totally incapable). Of these ten, only two shed any sperm at all, and this was rather watery. Preliminary experiments showed that when 3 drops of this watery "dry" sperm were added to about 10 c.c. of sea-water and about 8 drops of the resulting suspension were again diluted with 10 c.c. of sea-water, a suspension was obtained which caused mem-

brane swelling when 5 drops were added to 25 c.c. of sea-water, but only a moderate per cent. of fertilization when 1 drop was added to 25 c.c. of sea-water containing eggs. In the main experiment 3 drops of watery "dry" sperm were added to approximately 10 c.c. of sea-water, and the resulting suspension was the one used. Two Syracuse watch-crystals were employed. Watch-crystal *A* contained 4 c.c. of sea-water plus 1 c.c. of filtered blood (from ♀s). Watch-crystal *B* contained 5 c.c. of sea-water and no blood. 8 drops of a dilute egg suspension were then added to *A* and to *B* (at 11:15½ A.M.) and two minutes later, 2 drops of the sterm suspension just mentioned, were added. At 11:20, eggs in *B* (*i. e.*, in the absence of blood) all had membranes widely swollen all around. At 11:22, the eggs in *A* were examined. Most of the eggs showed not a trace of cortical change, but some showed membrane *elevation*. Shortly after, a count was made of eggs in *A*. It was found that 23 showed no cortical change, 1 was doubtful, it may have had a swollen membrane, 9 evidently possessed elevated membranes.

Thus the presence of blood prevents membrane swelling, and as one result of this prevention of excessive swelling, membrane elevation is possible, although without blood it could not have been produced. In this experiment, the anti-swelling effect of blood towards sperm suspension is conclusively demonstrated, for the blood evidently does not prevent access of sperm.[1]

The fact that the presence of blood inhibits membrane swelling in concentrated sperm suspensions, indicates that the swelling is produced by an acid. As is well known, all spermatozoa are abundantly provided with nucleic acid, and it is very probable that a nucleic acid or its derivative is responsible for the swelling.

I have tried a number of times to watch the process of normal membrane elevation under high power, but with only scant success. The presence of a coverslip produces difficulties. If a drop of egg suspension is placed on each of two slides, and one

[1] The further history of the eggs in this experiment is very interesting. On September 2, there are considerably fewer larvæ in *A* (where blood was present) than in *B*. But whereas all of the larvæ in *A* have well-marked arms, none of the larvæ in *B* possess even the suggestion of arms. On September 3, the larvæ in *B* are still perfectly armless, whereas those in *A* have the usual long arms of the pluteus stage.

drop is then covered by a coverslip with a drop of sperm suspension on its lower surface, and to the other drop is added a drop of the same sperm suspension, but no coverslip, a marked difference between the two preparations can be noted. In the absence of a coverslip, a much greater per cent. of eggs undergo membrane elevation. When the drop of sperm suspension is added to the eggs after the coverslip has been placed in position, practically no membrane elevation occurs. In these experiments, the coverslip was always supported by strips of paper or thin glass tubes, so that there could be no question of compressing the eggs.

The effect of the coverslip is in part due to the action of the glass (or of substances diffusing out of it)[1] on the spermatozoa. The sperm apparently congregate at the surface of the coverslip. But this is not believed to be the only effect, and some evidence that I possess, although not absolutely unimpeachable, tends to show that the pressure of the coverslip is also partly responsible for preventing elevation. However, further experiments on this point are necessary; I merely bring up the matter here in order to emphasize the difficulties in the way of direct observation. Fol ('79) speaks of the great difficulty in observing fertilization in the sea-urchin egg. Pictet ('91) found no such difficulty, but I think that the cortical effect that he describes was not membrane elevation, but membrane swelling, which is not retarded by the presence of the coverslip.[2]

My observations, though admittedly incomplete, tend to show that the membrane is elevated from all sides of the egg at the same moment. It is possible that elevation does start at the point of sperm entrance as Ries ('09) for example claims, but if this part of the membrane does show any priority, it is only an exceedingly brief one.

On the basis of Loeb's view that the sperm contains a lysin which produces membrane "formation" directly, we would have to suppose that this lysin diffuses around the egg surface in incredibly fast time. The morphology of sperm membrane elevation offers a severe obstacle to most theories which attempt

[1] Possibly dissolved out by the alkali of sea-water.

[2] Both Pictet and Fol subjected the eggs to slight compression.

to account for the process, but it is readily understandable on the basis of the surface tension theory. The membrane swelling which occurs at the point of sperm entrance, causes a lowering of surface tension not only in the immediate vicinity, but everywhere on the egg surface. Hence the vitelline membrane is lifted from all parts of the egg at practically the same moment.

G. The Significance of Cortical Change.

Although it has never been proven that cortical change is absolutely essential before development can take place, it is generally admitted that membrane "formation" must precede any normal development. We have seen that cortical change or membrane "formation" may involve either of two quite different processes. What then is the fundamental significance of cortical change, why is it necessary for normal development? I think that at least part of the answer is forthcoming. If an egg be watched under a micrometer scale, it will be noticed that shortly before the first cleavage the egg rapidly lengthens in the direction of its polar axes. In three minutes, an egg was in one case observed to increase its polar axis by 6.5μ, in another case by 8μ. (This fact is by no means new, all the pictures of cleavage show an increase in polar axis.) If the egg is surrounded by a stiff membrane, such a rapid change of form could scarcely be possible. But cortical change, whether it be membrane swelling or elevation, always results in the removal of this obstacle. The vitelline membrane is either rendered soft by swelling, or it is lifted away from the egg surface and its place taken by the no doubt less rigid hyaline layer. As a result, rapid changes in egg form can occur. Moreover, as is well known, the hyaline layer is normally pulled in between the first two blastomeres during the cleavage process. The stiff vitelline membrane could scarcely act in this way. But either membrane swelling or membrane elevation would result in the egg being invested by a membrane which was not too rigid to be pulled in. Thus at least two processes which play a part in normal development would be greatly hindered if some kind of cortical change did not occur.

Moreover when hypertonic solutions are used as reagents, the degree of rigidity of the plasma membrane becomes a factor of importance. As was shown previously (p. 158), exosmosis is favored by a cortical change which tends to soften the vitelline membrane.

III. Internal Change.—The Problem of Segmentation

A. Theories of Segmentation.

The real problem in the study of artificial parthenogenesis is not the problem of cortical change, but is rather the analysis of the factors which produce initiation of development. How can we define initiation of development? Although it has been shown that after fertilization there is a quickening of various energetic processes in the sea-urchin egg, plausible as such a view no doubt is, no one has ever shown that all the recorded instances of artificial parthenogenesis involve an increased metabolism. In practically every case, segmentation or mitosis has been regarded as the sole criterion of initiation of development. This is, I believe, wholly justifiable, as the act of segmentation is in itself a beginning of development.

Of the various theories of artificial parthenogenesis, those of R. Lillie and of Loeb have in recent years met with the most favor. The former believes that the initial change is an increase in permeability of the plasma membrane. This is the direct cause of (1) mitosis, and (2) increased metabolism. In his theory of mitosis (R. Lillie '11), now widely accepted as the least objectionable of any of the numerous theories of mitosis, he considers the hypothetical plasma membrane as charged by an electrical double layer; this charge is neutralized upon an increase in permeability. Such a neutralization of charge produces, according to Lillie, a lower potential at the surface as compared with the interior, and the difference in potential thus produced brings about a number of internal changes, which lead finally to the formation of a mitotic spindle. I confess that I am unable to understand the fundamental basis of the theory. It appears to me that any drop in the potential at the surface of a body involves *simultaneously* an exactly equivalent drop of the potential at every point in the interior. This seems

a direct consequence of the textbook definition of potential at a point, as the amount of work done by the electric field on unit charge, when it is brought to that point from infinity.

Moreover, even if we could accept some of the extremely dubious evidence that has been offered in favor of increased permeability, no one has ever tried to show that all parthenogenetic agents do cause increased permeability. In fact R. Lillie has at times assumed that hypertonic sea-water and that magnesium salts cause a decrease rather than an increase in permeability, and yet hypertonic sea-water, even in the presence of magnesium salts, produces initiation of development.

Of Loeb's ideas concerning the initiation of development only a bare outline is possible here. Recently, he is "inclined to believe that in all cases in which an unfertilized egg has been caused to develop a typical or atypical membrane had been formed."[1] This membrane "formation," which, as we have seen, Loeb regards as a swelling process, is the important initiative factor. It causes directly an increase of oxidations, but leaves the egg in a sickly condition, hence it is necessary to provide a corrective agent which may be either oxygen-containing hypertonic sea-water or the absence of oxygen. The theory of the necessity of the corrective factor need not concern us here, for we are primarily interested, not in the best method of obtaining artificial parthenogenesis, but rather in the nature of the physical or chemical change which causes *any* initiation of development. Loeb believes this change to be an increase in oxidations. In support of this view he has accumulated a mass of data. He first showed that in his original method of using a hypertonic solution alone, artificial parthenogenesis could only be produced in the presence of oxygen. More recently he has measured egg oxidations and has confirmed Warburg's observation that membrane "formation" produces a great increase in oxidations.

According to Loeb's measurements, any cytolytic change results in a great increase of egg oxidations.

B. The Action of Hypertonic Sea-water in the Presence of KCN.

Loeb was originally led to adopt the oxidation theory, by the fact that either the addition of KCN, or the removal of oxy-

[1] Loeb, '13*a*, p. 223.

gen by the passage of hydrogen, seemed to prevent the action of hypertonic solutions upon sea-urchin eggs. He reasoned from this that the hypertonic solutions produced an increase in oxidations. In 1913 and 1914, I performed a number of experiments with hypertonic solutions in the presence of KCN, or in an atmosphere of hydrogen. In this paper I shall, however, report only on the KCN experiments. The results obtained in an atmosphere of hydrogen are perhaps more interesting, but I do not feel as yet that every possible source of error has been eliminated.

At present it is generally accepted by most physiologists that KCN suppresses cell oxidations.[1] Loeb states that 2 c.c. 1/20 per cent. KCN to 50 c.c. of sea-water suffice for the purpose.[2] In my experiments, a much higher concentration of the reagent was employed.

On August 19, 1913, I added 0.5 c.c. of 1 per cent. KCN to every 58 c.c. of "NaCl hypertonic sea-water." (This addition of only 0.5 c.c. of more concentrated reagent had the advantage of not incurring as much dilution as the addition of 2 c.c. of almost pure solvent.) Eggs were exposed to this cyanide-containing "NaCl hypertonic sea-water," as well as to the normal "NaCl" hypertonic sea-water. The results appear in the folfowing table, in which the numerators of the fractions indicate the number of eggs observed to segment, the denominators the total number of eggs counted. Before return to sea-water, the eggs which had been exposed to KCN were washed twice by Lyon's test-tube method (Lyon '02), so that all trace of the poison might be removed.

Minutes Exposed.	"NaCl Hypertonic Sea-water."	"NaCl Hypertonic Sea-water" plus KCN.
25	127/209	10/102
30	83/100	9/108
35	62/100	22/247

Of the eggs exposed 25 or 30 minutes to the cyanide-containing hypertonic solution, none went beyond the 2 (or 3)-celled stage, but of the 22 eggs which segmented after an exposure of 35 minutes, two became many-celled blastulæ and may have gone

[1] Personally I do not believe that the evidence in support of this view is conclusive.

[2] Loeb, '09, p. 55.

farther, one reached a few-celled blastula stage. The control of unfertilized eggs in sea-water showed practically no segmentation, a count showed three out of more than five thousand.

In the above experiment only about 30–40 per cent. of the eggs underwent membrane elevation. In other experiments, in which less concentrated solutions of KCN were employed, all cortical change was inhibited and the eggs did not segment, although nuclear division sometimes occurred. In order to obtain the best results, widely elevated membranes must be obtained. If the membranes were only lifted a short distance from the egg in the cyanide-containing hypertonic sea-water, on a return to normal sea-water the egg expanded until it again came into contact with the membrane (often it ruptured the membrane and an exovate was produced). The best results were obtained in an experiment of September 2, 1914. In this case, 1 c.c. of 1/5 per cent. KCN was added to 24.5 c.c. of sea-water containing eggs, at 11:45 A.M. At 11:45 1/6 A. M., 4 c.c. of 2.5 *M* NaCl were added, so that the eggs were then in "NaCl hypertonic sea-water" plus KCN. They were allowed to remain in this solution for 33 minutes, during which time it was noticed that ninety-seven per cent. underwent membrane elevation. At 12:18 P.M., some eggs in 4 c.c. of the solution were transferred to about 600 c.c. of sea-water in a tall graduate. At about 12:45 P. M., most of the sea-water was siphoned off from the graduate and fresh sea-water added. At 2:30 P.M., out of 233 eggs examined 22 were found to have segmented, but the count was evidently too low, as doubtful cases were rejected. In some instances, exovates simulated cleavage. Only those eggs in which a nucleus could be observed in each cell were counted. At 4:55 P.M., 50 eggs, out of 110 examined, were found to have segmented, but this count was likewise probably too low. At 9 P.M., hundreds of motile blastulæ could be observed. (Of 337 eggs noted, 28 were motile larvæ, of these 18 swam on the bottom, 10 on the top.)

These experiments indicate that the action of the hypertonic solutions in initiating development is not due to an effect on the oxidative processes, for the hypertonic solution has the same action in the presence of a concentration of KCN, which ac-

cording to Loeb is considerably above that sufficient to check oxidations.

Loeb also found that the presence of KCN prevented degenerative change in eggs exposed to hypertonic sea-water, and assumed that the KCN acted by retarding excessive oxidations. However on the basis of our knowledge concerning the anti-swelling effect of KCN (*cf.* p. 174), it is simpler to assume that the inhibition of membrane swelling is the prime cause in preventing disintegration by the hypertonic solution.

The main support of the oxidation theory no doubt lies in the actual measurements of oxidations made by Warburg and Loeb. The method by which these measurements were made has been criticized in a note in *Science* (Heilbrunn '15).

C. An Analysis of the Methods of Producing Segmentation in the Unfertilized Arbacia Egg.

Hitherto in the study of artificial parthenogenesis, the general tendency has been to find new and different methods, rather than to find points of resemblance in the various means employed to produce segmentation in any one egg. The result has been that too much emphasis has been placed on the diversity and the unrelated character of the numerous parthenogenetic agents.

As a matter of fact there are in general only two ways of producing segmentation in the *Arbacia* egg, the endosmotic method and the exosmotic method. Whenever a reagent lowers the surface tension of the plasma-membrane, endosmosis is the result. The theoretical reason for this has already been considered (*cf.* p. 168). All those reagents which induce either true membrane elevation or membrane swelling are thus included in this category. In the first case the reagent produces a lowered surface-tension directly, in the second case, the swelling of the membrane results in a lowering of tension. Practically all of the reagents which have been used in artificial parthenogenesis produce either the one type of cortical change or the other. As a result, endosmosis follows, unless the eggs are in a hypertonic solution. In the latter case, water is extracted from the cell, and exosmosis occurs.

An apparent exception is found in the action of cold, which

has been stated to produce parthenogenesis in the sea-urchin egg (McClendon '10). Lowered temperatures should result in an increase, rather than a decrease of surface tension, nor can they be thought of as causing exosmosis.

In the only experiment tried, I found that instead of producing artificial parthenogenesis, lowered temperatures tended to retard the natural parthenogenesis which is usually manifested by the Arbacia egg. On June 25, the temperature of the aquarium in which the sea-urchins were kept, was 19.5°, the temperature of the sea-water as it emerged from the tap was 19°; the room temperature (at 4.45 P.M.) was 24.5°. At 10.40 A.M., a beaker containing eggs in a small amount of sea-water was placed in a large beaker, and the space intervening between the two beakers was filled with cracked ice. The beakers were then placed in the ice-box. At 11.00 A.M., the temperature of the sea-water surrounding the eggs was 3.5°, at 11.35 A.M. it was 1.5°, at 2.15 P.M. it was 0.5°, and at 4.15 P.M. it was 1.5°. Eggs were transferred from cold to normal sea-water after $3\frac{1}{2}$ hours (2.15 P.M.), 5 hours (3.40 P.M.), and $6\frac{1}{2}$ hours (5.05 P.M.). We can refer to these three lots of eggs as lot *A*, lot *B*, lot *C*, respectively.

Lot *A* when counted at 4.30 P.M. showed one doubtful case of segmentation, out of 100 eggs observed.

Lot *B* at 5.30 P.M., out of 1,000 eggs counted, showed 3 eggs apparently cleaving irregularly, and 1 doubtful case. At best 4/1000.

Lot *C* at 8.55 P.M. showed 9/1000 cleavages.

The control of untreated eggs (from the same female) at 4.40 P.M. showed, of 1,100 eggs counted, 4 eggs cleaving irregularly (1 a 7-celled stage) and 5 with attempted or incomplete cleavages. At 9.05 P.M. however, the control showed a much higher count. Of 526 eggs counted, 36 showed irregular divisions. Thus of the eggs counted at about 9.00 o'clock, those which had been exposed to cold for $6\frac{1}{2}$ hours showed less than 1 per cent. of segmenting eggs, whereas the control showed over 6 per cent. Thus the cold evidently retarded the process of natural parthenogenesis.

Of the two general methods of obtaining parthenogenesis, the exosmotic method yields the better results. Usually the endosmotic method produces only a small per cent. of segmenting eggs. But this is probably due to the specific poisoning action of the substances used in lowering surface tension, for when the sea-water is simply diluted, or when a harmless substance like egg albumen is used, much higher per cents. of dividing eggs are obtained. For example, when eggs were subjected to the action of a 1 per cent. solution of egg albumen (Kahlbaum) in sea-water, more than half of them divided, as shown in the accompanying table. In such a solution, the eggs were observed to undergo membrane swelling (see p. 173) and this was followed by endosmosis as shown by the increased diameter.

Length of Exposure to 1 Per Cent. Egg Albumen.	Segmentations.
30 minutes	55/100
66	48/100
118	50/100
233	6/100

The results gained with the endosmotic method used alone, are never as good as those which can be obtained with the exosmotic method. Neither are the per cents. of segmentation as high, nor is the degree of development attained as great. In spite of the fact that Loeb ascribes to hypertonic solutions a mere correcting effect, it is I think a noteworthy fact that no method of artificial parthenogenesis yet tried on the *Arbacia* egg, is truly effective, unless at some stage of the process it requires a hypertonic solution. In connection with this point, I made a number of experiments to test the butyric acid-KCN method which Loeb found so effective for *Strongłocentrotus*. I was at the time convinced that the method was essentially the same as Delage's acid and alkali method (Delage '07), and I tried to find if NaOH could not be substituted for the KCN, in other words if the correcting action of the latter was not due solely to its alkalinity. In no case, however, was I able to get any results either with KCN or NaOH as a "correcting agent" after butyric acid treatment. Indeed recently Loeb ('13*b*) has pointed out that the method is not suited for the *Arbacia* egg.

The question now arises if the two methods of producing segmentation, the one endosmotic, the other exosmotic, have anything in common. As a result of my experiments, I find that both methods produce a gelatinization or coagulation within the egg.[1]

Most biologists believe that the mitotic spindle is a condensation or coagulation product, and there is excellent support for this view. Hence any initiation of development must soon

[1] In the test-tube, gelatinization and coagulation of proteins are apparently quite different phenomena, the former converting the entire mass into a jelly, the latter resulting in a separation of a precipitate. But in the small field of action of a sea-urchin egg, it would not be so easy to distinguish between the two, for the entire egg is smaller than a single flake of the usual precipitate. Moreover, even in the test-tube coagulation is usually preceded by a stage strictly comparable to gelatinization; the entire mass becomes opalescent and assumes a greatly increased viscosity; only later does the precipitate appear.

lead to some coagulation before mitosis can be accomplished. At present all students of artificial parthenogenesis, if they consider this coagulation at all, regard it as a secondary result. Thus, some think an increase of oxidations is first produced by all parthenogenetic agents, and that the increased oxidation involves changes which produce coagulation. Others think of the prime cause as an increase of permeability. But it is also possible to believe that the primary effect of all the parthenogenetic reagents is a coagulation effect. This view has had as its adherents, at one time or another, some of the foremost students of artificial parthenogenesis. In his earliest papers on the subject, Loeb occasionally leaned toward coagulation as a possible primary effect, but he soon abandoned the idea to become its vigorous opponent. Delage for many years maintained that artificial parthenogenesis is the result of a coagulation followed by a liquefaction, he considered membrane elevation as one evidence of such a coagulation. At present, however, (Delage and Goldschmidt '13), he favors the Lillie theory of increased permeability as affording a more probable explanation of the facts. Possibly the most vigorous and scientific attempt to support the coagulation theory was that of Fischer and Ostwald ('05). These workers argued from a theoretical standpoint that all parthenogenetic agents are of such a nature as to produce coagulation. Later Ostwald ('07) retreated from this view and admitted that coagulation might be only secondarily produced as a result of increased oxidation. That all parthenogenetic agents cause coagulation, was denied by Loeb.[1] He pointed out that benzol, toluol, and saponin are not protein coagulants. Since then the theory of Fischer and Ostwald had had no one to defend it.

The view that I would maintain is that the only physicochemical effect which all parthenogenetic agents possess in common is the production of a gelatinization or coagulation within the egg. Hence I regard this gelatinization (or coagulation) as the direct cause of the initiation of development.

Leaving theory aside, it is possible to demonstrate that all parthenogenetic agents actually do produce gelatinization or

[1] Loeb, '09, p. 217.

coagulation within the egg. The distinguishing feature of a gel as opposed to a sol is its greater viscosity. All other macroscopic differences depend upon this. Indeed, according to Freundlich, the viscosity of a colloidal solution may be taken as a measure of its tendency to gelatinize ("Gelatinierungsbestreben").[1] The viscosity of the *Arbacia* egg protoplasm was regarded as an index of the state of aggregation of its constituents.

There are two general methods of measuring viscosity. One can either measure the rate of flow of a fluid, or one can study the movement of particles through the fluid. Both of these methods were used in studying the viscosity of the *Arbacia* cytoplasm. It is very easy to observe the rate of flow of the egg cytoplasm. One has only to exert pressure on the coverslip and the vitelline membrane soon bursts, allowing the egg contents to flow out. With this method, only great changes in viscosity can be noted, but gelatinization always does produce a very great increase in viscosity, so that the method usually suffices. The pressure can be applied by pushing on the coverslip with the point of a dissecting needle, or if greater accuracy is desired, a square piece of glass (broken from a slide) can be dropped from a known distance. This method of observing changes in viscosity is not entirely above criticism; for cortical changes, produced by the reagents used, might have an effect on the size of the aperture through which the cytoplasm flows.

The second method is much more exact and reliable. It involves a study of the movement of granules through the cytoplasm. The force which must be exerted to push the granules through the cytoplasm is a measu e of the viscosity of the latter. Centrifugal force is of necessity used, and a hand centrifuge does very nicely.[2] Into one tube of the centrifuge are placed eggs which have been treated in various ways; into the other, normal eggs. After the centrifuging has been accomplished, a microscopic examination reveals any differences which

[1] Freundlich, '09, p. 416. Freundlich and Ishizaka, '13.

[2] A Bausch and Lomb instrument was used in my experiments, and the eggs were placed into the small glass tubes of the hæmatocrit attachment. New tubes were used in each experiment so that all danger of contamination was avoided. One turn of the high-speed handle involved 130 revolutions of the tubes. The distance between the end of each tube and the axis was approximately $7\frac{1}{2}$ cm.

may exist between normal and treated eggs. When normal unfertilized *Arbacia* eggs are centrifuged vigorously, the protoplasmic materials rapidly become separated into four layers or zones. Lyon ('07) has given an excellent description of the appearance of these zones and the reader is referred to his paper for detai s. The pigment-bearing granules come to be all massed at one pole, in the pigment zone; next to this is a zone of granular material, then a hyaline zone, and at the pole opposite the pigment zone is a small dense accumulation of substance which because of its color is known as the gray cap. The egg nucleus lies in the hyaline zone, directly beneath the gray cap. When an egg shows all these zones, I shall refer to it as "stratified." As the viscosity of the protoplasm increases, stratification becomes more and more difficult; in a thoroughly coagulated egg no stratification is possible.

Hypertonic solutions produce a very noticeable coagulative change in the sea-urchin egg. In 1913 a few preliminary experiments were performed in which the eggs were pressed out of shape by pushing down on the coverslip with a dissecting needle. It was found that "NaCl hypertonic sea-water" produced a marked increase in the viscosity of the cytoplasm. Such a solution causes swelling of the vitelline membrane. This swelling is apparently absent if a freshly prepared 0.49 *M* $MgCl_2$ solution is used. Eggs treated with this solution became very much more viscous than they had been previously. Whereas the normal unfertilized eggs shot out their contents rapidly if subjected to a slight pressure, eggs which had been immersed in $MgCl_2$ for 85 minutes could be subjected to considerable pressure without losing their circular outline. They could indeed be flattened out into a thin "pancake."

More accurate data were obtained with the centrifuging method. On July 23, 1914, some eggs were placed into 50 c.c. of sea-water plus 8 c.c. of 2.5 *M* NaCl at 2:21¾ P.M. At 2:44 P.M., these eggs were placed into one tube of the centrifuge, and into the other tube were placed some normal untreated eggs of the same female. At 2:45 P.M., after a few preliminary turns, the tubes were revolved for 28 seconds at a rate of 162 revolutions per second. The eggs were then examined. The

normal eggs showed the typical zones of centrifuged eggs. The pigment zone extended for about one fourth of the egg diameter along the axis of stratification. The contrast between normal eggs and those which had been treated with the hypertonic solution was very marked. The latter as a rule showed no stratification whatsoever, although some eggs showed just the beginnings of such a process, the pigment granules being slightly more abundant at one pole of the egg than at the pole opposite to it.

The experiment was repeated on July 24. In this case unfertilized eggs were subjected to "NaCl hypertonic sea-water" at 10:29 A.M., and about 25 minutes later they were placed into one tube of the centrifuge. Into the other tube, normal eggs were placed. Then (at 10:55½ A.M.) the tubes were revolved for 19 seconds at the rate of 171 revolutions per second. Upon examination, the normal eggs showed complete stratification under low power of the microscope. High power examination showed that a few pigment granules had not reached the pigment zone, but had lingered near the equator of the eggs. The eggs treated with hypertonic sea-water showed no stratification. In some eggs one pole was slightly paler than the other, and fewer pigment-bearing granules could be found at this pole. But even in these eggs, the pigment was scattered throughout every part of the cytoplasm. At 11:29 the eggs in hypertonic sea-water were centrifuged again and compared with normal eggs given the same treatment. Longer and more vigorous centrifuging was resorted to, and the tubes of the centrifuge were revolved 186 times per second for 35 seconds. The normal eggs now appeared perfectly stratified; they were often elongated as a result of the treatment. The eggs in hypertonic sea-water usually showed only the beginnings of stratification, a tendency for the pigment to be massed toward one pole. In a few eggs, however, the pigment was practically limited to one half of the egg.

These experiments show a striking increase in cytoplasmic viscosity after eggs have been exposed to hypertonic solutions. This could be noted both by observation of the flow of the cytoplasm itself, as well as by more careful observations of the

movements of granules through the cytoplasm. The only explanation of such a marked increase in viscosity is that gelatinization or coagulation has taken place within the egg. Undoubtedly exosmosis causes some constituent of the cytoplasm to change from sol to gel.

Endosmosis was also found to cause coagulation, either when it resulted from a dilution of the outer medium, or when it was the result of a lowered surface tension of the vitelline or plasma membrane. This could be demonstrated either by observing the cytoplasmic flow from compressed eggs, or by noting the granular movements in centrifuged eggs. Numerous experiments were made with distilled water. In an experiment of June 30, 1914, a square piece of glass which had been broken from a slide was used to compress the eggs. It weighed 1.31 grams. The piece of glass was held between the thumb and forefinger, so that its one edge rested on the slide just to one side of the coverslip which covered the eggs under observation. At the desired time, the glass piece was dropped and the compressed eggs could then be immediately observed. Normal eggs were deprived of their jelly by shaking them 6 or 8 times in a test-tube, and were then subjected to the pressure of the piece of glass as just described. The eggs all ruptured, the contents flowing out for a distance of about 60μ. Eggs (from the same female) were then dropped into distilled water at 10:28 A.M. At 10:28$\frac{3}{4}$ any jelly which may have remained around them was removed by shaking the eggs. At 10:29$\frac{1}{2}$ the eggs were subjected to the pressure of the same piece of glass which had been used in the case of the normal eggs. They resisted the pressure and remained circular or nearly circular in outline. In the eggs observed, membrane elevation had not taken place. The greater resistance to pressure of the eggs treated with distilled water is an indication of their increased viscosity. The eggs tended to remain spherical, they showed a definite elasticity. Similar observations showing increased viscosity after treatment with distilled water, were made a number of times both in 1913 and 1914.

On July 25, 1914, at 3:59 P.M., 7 drops of an egg suspension were dropped into 15 c.c. of distilled water. The eggs in the

distilled water were then hastily pipetted into one tube of the centrifuge, the other tube contained untreated eggs. The centrifuge was started at 4:00½ P.M., and for 25 seconds the tubes were revolved at a rate of 156 revolutions per second. The eggs in distilled water were examined as soon as possible (about half a minute later). They showed not a sign of stratification. Most of the pigment had been lost as a result of the action of the distilled water, but high power examination showed that the pigment-bearing granules still retained some pigment. These granules were scattered all through the cell. On the other hand the normal eggs showed the typical stratification. The viscosity of the cytoplasm had therefore increased enormously in the eggs treated with distilled water, so that the granules were prevented from wandering through it. In another experiment (July 22, 1914) the eggs were transferred back to sea-water before being centrifuged. At 3:51 P.M., 5 drops of egg suspension were added to 15 c.c. of distilled water. At 3:55 P.M., some of these eggs were removed from the distilled water and placed into sea-water again. The eggs thus transferred were the ones studied; they were placed into one tube of the centrifuge, normal eggs occupying the other. Beginning at 3:59½ P.M., the tubes were revolved 165 times per second for 30 seconds. Upon examination it was found that whereas the normal eggs were completely stratified, not a sign of stratification could be observed in the eggs which had been exposed to distilled water. Evidently endosmosis, following immersion in distilled water, leads to a gelatinization or coagulation in the cytoplasm.

This effect is also produced when endosmosis follows a lowered surface tension of the plasma membrane. A drop of egg suspension was stirred up with a drop of chloroform, and the eggs rapidly increased in diameter. When subjected to the pressure of a piece of glass broken from a slide, the eggs flattened out but remained perfectly circular in outline. The same result was obtained if toluol was used instead of chloroform. The eggs under pressure behave like eggs which have been coagulated by some typical coagulant, *e. g.*, $HgCl_2$. On the other hand, when the normal eggs were subjected to the pressure of the same piece of glass, the cytoplasm flowed out a considerable distance. The

results obtained with the centrifuging method are still more convincing.

At 10:02½ A.M. (July 23, 1914), 2.5 c.c. of toluol were added to 2.5 c.c. of sea-water containing eggs, and the mixture was stirred thoroughly with a glass rod (in a Syracuse watch-crystal). At 10:07½ A.M., 7 drops of the egg-containing liquid were transferred back to normal sea-water. Into one tube of the centrifuge were placed normal eggs, the other contained eggs which had been treated with toluol for five minutes. The eggs were then centrifuged for 30 seconds at a rate of 152 revolutions per second. Upon examination, the normal eggs appeared perfectly stratified, whereas those which had been exposed to toluol showed not a trace of stratification. As a result of the toluol treatment the eggs increased their diameter about 2μ. The experiment was repeated with identical results on July 25, 1 c.c. of toluol being added to 3 c.c. of sea-water. In this case the eggs were exposed 5 minutes, and were then centrifuged 23 seconds at an average of 158 revolutions per second.

Saponin was also found to produce a coagulation within the egg. At 5:35 P.M. (July 23), 7 drops of an egg suspension were placed in about 15 c.c. of 0.2 per cent. saponin solution (in sea-water). At 5:40 P.M., the eggs were returned to normal sea-water. The eggs were then subjected to an exceptionally long centrifuging process. Beginning at 5:47 P.M., for 50 seconds they were revolved at an average of 166.5 revolutions per second. Normal eggs subjected to this centrifuging process were of course completely stratified. Of the eggs treated with saponin, only about 15–20 per cent. showed any signs of stratification, the remaining eggs were all totally unstratified.

In the experiments with saponin and toluol, the reagent produces a lowering of surface tension directly. With some reagents, membrane swelling occurs first, and the lowered surface tension thus produced results in endosmosis. In this case also, coagulation follows an increase in egg volume. On August 4, 1914, eggs were washed in 0.55 *M* NaCl solution and were then dropped into 0.55 *M* NaCl plus 1.5 c.c. *N*/10 NaOH at 10:59¼ A.M. These eggs were then placed into one tube of the centrifuge, and into the other were placed eggs which had been im-

mersed in 25 c.c. of 0.55 *M* NaCl at 10:58 A.M. Beginning at 11:16½ A.M., the eggs were centrifuged for 17 seconds at the rate of 153 revolutions per second. When examined, the eggs in NaCl alone showed stratification, although the boundary between the various zones was not a sharp one. Gray cap, hyaline zone, granular zone, were distinguishable, but the pigment was not entirely restricted to the pigment zone. On the other hand, the eggs in the alkaline NaCl solution showed not a vestige of stratification, in every case the pigment-bearing granules were evenly distributed throughout the cell. In these eggs exposed to an alkaline NaCl solution, membrane swelling had taken place. Similarly when membrane swelling was induced by 1 per cent. albumen, coagulation followed the endosmosis thus produced. On August 4, 1914, at 10:40 A.M., some eggs were placed in a filtered 1 per cent. solution of egg albumen solution in sea-water. After about 50 minutes, some eggs in the albumen solution were put into one tube of the centrifuge, normal eggs were placed in the other. The eggs were then centrifuged at the rate of 143 revolutions per second for 20 seconds. The normal eggs all showed stratification, although the various zones were not sharply marked off from each other. Of the eggs in the albumen solution only a few were examined. Of these ten showed not a trace of stratification, one egg showed stratification, but on examination it was found that its membrane had remained unswollen. Pressure experiments also showed a great increase in viscosity after membrane swelling. Sodium iodide and 1 per cent. albumen were used in these experiments.

It is evident that both the reagents which cause exosmosis and those which produce endosmosis, cause gelatinization or coagulation of some substance in the *Arbacia* egg. Thus all parthenogenetic agents produce such coagulative change.[1] The experiments indicate that the coagulation occurs just as

[1] I have omitted mention of radium and ultra-violet rays as parthenogenetic agents. I have not worked with either of these methods, and so can only offer a theoretical interpretation. Judging from Loeb's description (*Science*, N.S., XL., 680 (1914)), ultra-violet rays produce membrane swelling and thus they no doubt induce endosmosis. Moreover both radium and ultra-violet rays are protein coagulants, according to Dreyer and Hanssen, C. R. Acad. Sci., CXLV., 234 (1907). Hardy has also decribed gelatinization of a globulin solution as a result of radium radiation. Proc. Camb. Phil. Soc., XII., 201 (1903).

soon as water enters or leaves the cell. It is probable therefore that the effect is primary, and not the result of intermediate changes.

In normal fertilization, the sperm likewise produces coagulative changes in the cytoplasm. Albrecht in 1898 showed that there was an increase in viscosity after fertilization. He drew his conclusions from observations on compressed eggs. I have repeated Albrecht's observations a number of times. Much more striking demonstration of gelatinization or coagulation after fertilization is afforded by the centrifuge method. On July 22, 1914, eggs from a single female were washed twice and divided into two lots. At 10:00 P.M., half of the eggs were fertilized. One tube of the centrifuge was then filled with fertilized eggs, the other contained unfertilized eggs. At 10:10 P.M., the tubes were revolved for 18 seconds at an average speed of 180.5 revolutions per second. The eggs were then examined immediately. The unfertilized eggs were typically stratified. On the other hand, the eggs which had been fertilized were not at all stratified. Out of hundreds examined, only one showed stratification and that was peculiar in lacking an elevated membrane. Probably it had escaped fertilization. The coagulative change begins to be apparent very soon after fertilization. In one case the beginnings of the process could be observed $2\frac{1}{2}$ minutes after insemination. At 8:24 P.M. (July 26) some eggs were fertilized in a small volume of sea-water and were shaken about to insure rapid sperm contact. At 8:26$\frac{1}{2}$ P.M., fertilized and unfertilized eggs were centrifuged at the same time (in separate tubes). For 23 seconds an average speed of 141 revolutions per second was maintained. Upon examination the fertilized eggs showed some evidences of stratification. The gray cap was becoming evident in some. But most of the fertilized eggs showed little more than a tendency for the pigment to mass at one pole. The unfertilized eggs were clearly more stratified. They showed the gray cap plainly in all cases, and a hyaline zone was also recognizable in them. Thus only $2\frac{1}{2}$ minutes after insemination, the sperm had already begun to produce coagulative changes in the cytoplasm.

From these experiments, I have been led to conclude that

initiation of development, either artificially produced or the result of fertilization, always involves a gelatinization or coagulation with the egg. This coagulative change is evoked before the egg interior shows any other signs of the approach of development. The coagulating substance (or substances) is evidently very ready to coagulate. The ensemble of conditions within the egg is no doubt responsible for this unstable state. Only a slight change in salt concentration is then sufficient to bring about coagulation. The fact that both increase and decrease of salt concentration are effective, suggests that the protein involved is of the globulin type.[1]

The mitotic spindle probably arises as a direct result of the coagulative change. The actual explanation of how this occurs is not truly a problem of biology but a problem of colloid chemistry, for it has been shown (Fischer, '99) that coagulation of inanimate proteins can produce structures identical in appearance with the mitotic spindle.

IV. Summary.

1. The unfertilized *Arbacia* egg consists essentially of fluid proteins, surrounded by a stiff membrane (the vitelline membrane), which is the plasma membrane of the egg cell. This membrane is a protein gel with little or no admixture of lipoids.

2. There are two types of cortical change, membrane swelling and membrane elevation. In the former, the membrane absorbs water and increases in thickness; in the latter, the normal process, it becomes lifted away from the egg surface.

3. The vitelline membrane only loses its semipermeable properties several minutes after elevation, and the increase of permeability that then ensues is best regarded as a result rather than as a cause of the process.

4. Artificial membrane elevation is produced only by substances which lower the surface tension of the vitelline membrane. This is explainable on the basis of a theory that considers the various forces at play on the membrane. After its surface tension is lowered, the forces exerted outward are stronger than

[1] If the protein referred to here is, as seems most likely, the substance which forms the spindle fibers, the fact that it shows the properties of a globulin becomes of significance. For globulins are the proteins most closely associated with contractile processes, muscles consisting almost entirely of them.

those balancing them, and the membrane is as a result pushed away from the egg.

5. Cytolysis is not due to a simple swelling of egg proteins for these can be shown to coagulate rather than to swell, it is due to a continuation of the same process as that which results first in membrane elevation.

6. The sperm produces membrane elevation by lowering the surface tension of the vitelline membrane. This it accomplishes by causing the membrane to swell at the point where it strikes the egg.

7. Experimental evidence is presented to show that this membrane swelling produced by the sperm is due to the action of an acid.

8. The fact that potassium cyanide does not prevent initiation of development by hypertonic sea-water, is taken as evidence against the oxidation theory of artificial parthenogenesis.

9. All initiation of development involves the gelatinization or coagulation of some substance within the egg. This coagulative change can be demonstrated to take place before the egg interior shows any other signs of the approach of development.

The above summary covers only the more important points of the paper.

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V. References.

Albrecht, E.

'98 Untersuchungen zur Struktur des Seeigeleies. Sitzungsber. d. Ges. f. Morph. u. Physiol. in München, 1898, pp. 133–141.

Czapek.

'11 Ueber eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen, Jena, 1911, G. Fischer.

Delage, Y.

'07 L'oxygène, la pression osmotique, les acides et les alcalis dans la parthénogenèse experimentale. C. R. Acad. Sci. Paris, T. 145, pp. 218–224.

Delage, Y. and Goldschmidt, M.

'13 La parthénogenèse naturelle et expérimentale, Paris, 1913.

Fischer, A.

'99 Fixirung, Färbung und Bau des Protoplasmas, Jena, 1899, G. Fischer.

Fischer, M. H.

'10 Oedema. New York, Wiley.

'12 A Further Response to Some Criticisms of the Colloid-chemical Theory of Water Absorption by Protoplasm. Journ. Amer. Med. Assn., Vol. 59, pp. 1429–1433.

Fischer, M. H. and Ostwald, Wolfgang.

'05 Zur physikalisch-chemischen Theorie der Befruchtung. Arch. f. d. ges. Physiol., Bd. 106, pp. 229–266.

Fol, H.

'79 Recherches sur la fécondation et le commencement de l'hénogénie chez divers animaux. Mem. d. l. Soc. de Phys. et d'Hist. Nat. de Genève, T. 26.

Freundlich, H.

'09 Kapillarchemie, Leipzig, 1909.

Freundlich, H., and Ishizaka, N.

'13 The Velocity of Coagulation of Aluminium Hydroxide Sols as Measured by Changes in the Viscosity. Trans. Faraday Society, Vol. 9, pp. 66–79.

Gibbs, J. Willard.

'75–'78 On the Equilibrium of Heterogeneous Substances. Republished in the Scientific Papers of J. Willard Gibbs, London and New York, 1906.

Heilbrunn, L. V.

'13 Studies in Artificial Parthenogenesis—I. Membrane Elevation in the Arbacia Egg. Biol. Bull., Vol. 24, pp. 345–361.

'15 The Measurement of Oxidations in the Sea-urchin Egg. Science (in press).

Herxheimer, G.

Mikroskopische Technik, in Abderhalden's Handbuch der biochemischen Arbeitsmethoden, Bd. VII., pp. 632–714 (see pp. 679–680).

Kite, G. L.

'13 Studies on the Physical Properties of Protoplasm. I. The Physical Properties of the Protoplasm of Certain Animal and Plant Cells. Amer. Journ. Physiol., Vol. 32, pp. 146–164.

Lepeschkin, W. W.

'11 Zur Kenntnis der chemischen Zusammensetzung der Plasmamembran. Ber. d. deutsch. bot. Ges., Bd. 29, pp. 247–261.

Lillie, Ralph S.

'10 The Physiology of Cell Division—II. The Action of Isotonic Solutions of Neutral Salts on Unfertilized Eggs of Asterias and Arbacia. Amer. Journ. Physiol., Vol. 26, pp. 106–133.

'11 The Physiology of Cell Division—IV. The Action of Salt Solutions Followed by Hypertonic Sea-water on Unfertilized Sea-urchin Eggs and the Rôle of Membranes in Mitosis. Journ. Morph., Vol. 22, pp. 695–730.

Loeb, Jacques.

'08 Ueber den Mechanismus der Agglutination. Kolloid-Zeitsch., Bd. 3, pp. 113–114.

'09 Die chemische Entwicklungserregung des tierischen Eies, Berlin, 1909, Springer.

'13a Artificial Parthenogenesis and Fertilization. Chicago, 1913, University of Chicago Press.

'13b Reversibility in Artificial Parthenogenesis. Science, N.S., Vol. 38, pp. 749–751.

Loewe, S.

'13 Membran and Narkose. Weitere Beiträge zu einer Kolloidchemischen Theorie der Narkose. Biochem. Zeitschr., Bd. 57, pp. 161–260.

Lyon, E. P.

'02 Effects of Potassium Cyanide and of Lack of Oxygen upon the Fertilized Eggs and the Embryos of the Sea-urchin (*Arbacia punctulata*). Amer. Journ. Physiol., Vol. 7, pp. 56–75.

'07 Results of Centrifugalizing Eggs. Arch. f. Entwicklungsmech., Bd. 23, pp. 151–173.

McClendon, J. G.

'10 On the Dynamics of Cell Division—II. Changes in Permeability of Developing Eggs to Electrolytes. Amer. Journ. Physiol., Vol. 27, pp. 240–275.

Ostwald, Wolfgang.

'07 Über das Vorkommen von oxydativen Fermenten in den reifen Geschlechtszellen von Amphibien und über die Rolle dieser Fermente bei den Vorgängen der Entwicklungserregung. Biochem. Zeitsch., Bd. 7, pp. 409–472.

Pictet, C.

'91 Recherches sur la spermatogénèse chez quelques Invertebrés de la Méditerranée. Mitth. d. zool. Stat. z. Neapel, Bd. 10, pp. 75–152 (see pp. 100–105).

Quincke, G.

'77 Ueber Diffusion und die Frage, ob Glas für Gase undurchdringlich ist. Poggendorff's Annalen, Bd. 160, pp. 118–123.

'02 Ueber unsichtbare Flüssigkeitsschichten und die Oberflächenspannung flüssiger Niederschläge bei Niederschlagsmembranen, Zellen, Colloiden und Gallerten, Ann. d. Physik, 4te Folge, Bd. 7, pp. 631–682, 701–744.

Reinke, F.

'95 Untersuchung über Befruchtung und Furchung des Eies der Echinodermen. Sitzungsber. d. Akad. d. Wiss. z. Berlin, 1895, pp. 625–637.

Ries, J.

'09 Kinematographie der Befruchtung und Zellteilung. Arch. f. mik. Anat., Bd. 74, pp. 1–31.

Robertson, T. B.

'12 Studies in the Fertilization of the Eggs of a Sea-Urchin (*Strongylocentrotus purpuratus*) by Blood-Sera, Sperm-Extract and Other Fertilizing Agents. Arch. f. Entwicklungsmech., Bd. 35, pp. 64–130.